MYCOLOGIA

VOLUME XLI, 1949

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OBSERVATIONS ON STREPTOMYCES GRISEUS. III. CARBON SOURCES FOR GROWTH AND STREPTOMYCIN PRODUCTION [1]

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In a previous paper (1), the use of various compounds as nitrogen sources for growth and streptomycin production in a synthetic medium was discussed. Glucose always served as the carbohydrate in these experiments.

Thus far, little has been disclosed of the use of various carbohydrates in streptomycin fermentation mediums. The meat extract-peptone medium of Schatz, Bugie, and Waksman (5), the soy bean medium of Rake and Donovick (3), and the near synthetic medium of Vander Brook et al. (6) all contained glucose. Hubbard and Thornberry (2), using surface fermentations, tested a number of carbon sources. These compounds, both sugars and alcohols, were added to a basal medium containing ammonium lactate and mineral salts. Of the compounds tested, d-mannose, d-galactose, maltose, cellobiose, mannitol, and dextrin supported the highest yields. Saunders and Sylvester (4) also tested a few sugars and organic acids as constituents of a synthetic streptomycin fermentation medium. They employed submerged fermentations with a basal medium containing a sugar, an organic acid, an inorganic nitrogen compound, and mineral salts. Five sugars, namely, glucose, dextrin, brown sugar, starch, and lactose, were

[1] Contribution from the Research Laboratories of Merck & Co., Inc.

tested. Glucose, dextrin, and starch supported the highest yields. Of the organic acids tested, lactic and acetic gave the best results.

This paper concerns the possible substitution of a number of carbon sources, *i.e.,* sugars, alcohols, and organic acids, for glucose in synthetic streptomycin fermentation mediums.

MATERIALS AND METHODS

The experimental conditions were the same as those employed in the testing of various nitrogen sources in synthetic streptomycin fermentation mediums (1).

Cultures were developed by spreading spores from the soil tube previously used (1) over Blake bottle slants of yeast extract-glucose agar. After seven days' incubation at 28° C., fifty ml. of sterile distilled water were added to each Blake bottle culture and a spore suspension prepared. One ml. of this spore suspension was added to each fermentation flask.

The basal medium contained the following salts in one liter of distilled water: NaCl 5.0 gm., K_2HPO_4 2.0 gm., $MgSO_4 \cdot 7H_2O$ 1.0 gm., $CaCl_2$ 0.4 gm., $FeSO_4 \cdot 7H_2O$ 20.0 mg., and $ZnSO_4 \cdot 7H_2O$ 10.0 mg. The nitrogen and carbon sources were added to this salt solution. Because the metabolism of the carbohydrate is affected by the nitrogen source, each carbon source was tested in mediums containing three different nitrogen sources. These nitrogen compounds were employed at the following concentrations: $(NH_4)_2$-HPO_4 at 4.0 gm. per liter, enzyme-digested casein at 10.0 gm. per liter, and l(−)proline at 15 gm. per liter. The carbon sources were added to these three different mediums before sterilization and the pH adjusted, when necessary, to between 7.0 and 7.5 with 1 N NaOH.

The mediums were dispensed in 40-ml. amounts in 125-ml. Erlenmeyer flasks and autoclaved at 121° C. for 17 minutes. The pH was checked after autoclaving, and no medium with an initial pH below 6.5 was used. After inoculation, the flasks were incubated at 28° C. on a rotary-type shaker moving at 220 r.p.m. so that it described a circle one inch in diameter. After growth started, the flasks were sampled daily until after the maximum broth potencies were reached. The whole broth samples

were assayed daily by the agar plate method using streptomycin calcium chloride complex as a standard.

EXPERIMENTAL RESULTS

The carbon sources that were tested included sugars, alcohols, and organic acids. All of these compounds were added to the mediums at a 1.0 per cent level.

The streptomycin broth potencies produced in the mediums with various sugars serving as the carbon source are shown in table I. When alcohols were substituted for the sugars, the re-

TABLE I
SUGARS AS CARBON SOURCES

Sugar	Nitrogen Source	Maximum Streptomycin Broth Potency—γ/ml.	Day of Maximum
d-arabinose	$(NH_4)_2HPO_4$	very slight growth	
d-arabinose	casein digest	76	3
d-arabinose	l(−)proline	11	12
l-arabinose	$(NH_4)_2HPO_4$	very slight growth	
l-arabinose	casein digest	121	3
l-arabinose	l(−)proline	very slight growth	
fucose	$(NH_4)_2HPO_4$	no growth	
fucose	casein digest	99	3
fucose	l(−)proline	33	11
lyxose	$(NH_4)_2HPO_4$	poor growth	
lyxose	casein digest	150	3
lyxose	l(−)proline	very slight growth	
l-rhamnose	$(NH_4)_2HPO_4$	very slight growth	
l-rhamnose	casein digest	220	5
l-rhamnose	l(−)proline	13	9
d-ribose	$(NH_4)_2HPO_4$	10	8
d-ribose	casein digest	236	5
d-ribose	l(−)proline	very slight growth	
d-xylose	$(NH_4)_2HPO_4$	no growth	
d-xylose	casein digest	171	3
d-xylose	l(−)proline	no growth	
l-xylose	$(NH_4)_2HPO_4$	48	14
l-xylose	casein digest	183	3
l-xylose	l(−)proline	79	10
glucose	$(NH_4)_2HPO_4$	136	6
glucose	casein digest	439	4
glucose	l(−)proline	800	12
galactose	$(NH_4)_2HPO_4$	123	5
galactose	casein digest	369	4
galactose	l(−)proline	124	12
levulose	$(NH_4)_2HPO_4$	116	9
levulose	casein digest	260	4
levulose	l(−)proline	525	10
d(+)mannose	$(NH_4)_2HPO_4$	85	7
d(+)mannose	casein digest	221	3
d(+)mannose	l(−)proline	900	10

TABLE I—*Continued*

Sugar	Nitrogen Source	Maximum Streptomycin Broth Potency—γ/ml.	Day of Maximum
l-sorbose	$(NH_4)_2HPO_4$	very slight growth	
l-sorbose	casein digest	41	5
l-sorbose	l(—)proline	no growth	
cellobiose	$(NH_4)_2HPO_4$	54	5
cellobiose	casein digest	244	4
cellobiose	l(—)proline	347	10
lactose	$(NH_4)_2HPO_4$	108	5
lactose	casein digest	247	4
lactose	l(—)proline	588	11
maltose	$(NH_4)_2HPO_4$	140	8
maltose	casein digest	269	4
maltose	l(—)proline	800	11
melibiose	$(NH_4)_2HPO_4$	no growth	
melibiose	casein digest	146	4
melibiose	l(—)proline	272	9
sucrose	$(NH_4)_2HPO_4$	poor growth	
sucrose	casein digest	80	4
sucrose	l(—)proline	134	10
melezitose	$(NH_4)_2HPO_4$	very slight growth	
melezitose	casein digest	146	4
melezitose	l(—)proline	108	8
raffinose	$(NH_4)_2HPO_4$	very slight growth	
raffinose	casein digest	51	6
raffinose	l(—)proline	48	12
dextrin	$(NH_4)_2HPO_4$	74	7
dextrin	casein digest	235	4
dextrin	l(—)proline	630	8
inulin	$(NH_4)_2HPO_4$	no growth	
inulin	casein digest	112	3
inulin	l(—)proline	·69	11
starch	$(NH_4)_2HPO_4$	103	8
starch	casein digest	293	4
starch	l(—)proline	433	8
no sugar	casein digest	93	3
no sugar	l(—)proline	43	12

sults shown in table II were obtained. Of the fifteen organic acids that are listed in table III, only seven supported growth and streptomycin production. The results given in table III were obtained in the $(NH_4)_2HPO_4$ medium to which the organic acids were added.

It will be noted that in some instances growth is recorded as very slight. In appraising these results, one should remember that the inoculum was grown on yeast extract-glucose agar. Spores from such cultures were not washed before being added to the mediums. Perhaps enough organic material was transferred with the inoculum to allow slight growth.

TABLE II
ALCOHOLS AS CARBON SOURCES

Alcohol	Nitrogen Source	Maximum Streptomycin Broth Potency—γ/ml.	Day of Maximum
methanol	$(NH_4)_2HPO_4$	no growth	
methanol	casein digest	51	4
methanol	l(−)proline	2	10
ethanol	$(NH_4)_2HPO_4$	no growth	
ethanol	casein digest	75	3
ethanol	l(−)proline	2	10
propanol	$(NH_4)_2HPO_4$	no growth	
propanol	casein digest	53	4
propanol	l(−)proline	0	
butanol	$(NH_4)_2HPO_4$	no growth	
butanol	casein digest	55	3
butanol	l(−)proline	7	11
glycerol	$(NH_4)_2HPO_2$	82	11
glycerol	casein digest	156	4
glycerol	l(−)proline	176	10
erythritol	$(NH_4)_2HPO_4$	no growth	
erythritol	casein digest	22	4
erythritol	l(−)proline	11	10
pentaerythritol	$(NH_4)_2HPO_4$	no growth	
pentaerythritol	casein digest	38	4
pentaerythritol	l(−)proline	7	11
sorbitol	$(NH_4)_2HPO_4$	no growth	
sorbitol	casein digest	0	
sorbitol	l(−)proline	0	
dulcitol	$(NH_4)_2HPO_4$	no growth	
dulcitol	casein digest	37	5
dulcitol	l(−)proline	17	9
mannitol	$(NH_4)_2HPO_4$	162	10
mannitol	casein digest	242	4
mannitol	l(−)proline	700	10
inositol	$(NH_4)_2HPO_4$	no growth	
inositol	casein digest	60	3
inositol	l(−)proline	42	11
no alcohol	casein digest	93	3
no alcohol	l(−)proline	43	12

DISCUSSION

Sugars: None of the pentoses supported high yields of streptomycin. Only two aldopentoses, namely, d-ribose and l-xylose, supported streptomycin production in the medium containing an inorganic nitrogen source. The yields thus obtained were quite low, 10 γ/ml. and 48 γ/ml., respectively. Neither of the methylpentoses, namely, l-rhamnose or fucose, supported yields in the $(NH_4)_2HPO_4$ medium. In the medium containing enzyme-digested casein as the nitrogen source, the use of l-arabinose, lyxose, l-rhamnose, d-ribose, d-xylose, and l-xylose resulted in

TABLE III

ORGANIC ACIDS AS CARBON SOURCES

Organic Acid	Nitrogen Source	Maximum Streptomycin Broth Potency—γ/ml.	Day of Maximum
adipic	$(NH_4)_2HPO_4$	no growth	
acetic	$(NH_4)_2HPO_4$	no growth	
citric	$(NH_4)_2HPO_4$	9	8
fumaric	$(NH_4)_2HPO_4$	5	5
gallic	$(NH_4)_2HPO_4$	no growth	
gluconic	$(NH_4)_2HPO_4$	9	7
lactic	$(NH_4)_2HPO_4$	13	8
levulinic	$(NH_4)_2HPO_4$	no growth	
malic	$(NH_4)_2HPO_4$	8	5
malonic	$(NH_4)_2HPO_4$	no growth	
oxalic	$(NH_4)_2HPO_4$	no growth	
pimelic	$(NH_4)_2HPO_4$	no growth	
pyruvic	$(NH_4)_2HPO_4$	22	9
succinic	$(NH_4)_2HPO_4$	2	8
tartaric	$(NH_4)_2HPO_4$	no growth	

streptomycin titers significantly higher than were obtained in the casein medium with no sugar added. Perhaps some of the pentoses would support high yields if some specific amino acids served as the nitrogen source. With 1(—)proline as the nitrogen source, however, none of the pentoses except l-xylose resulted in yields higher than were obtained in the proline medium to which no sugar had been added.

All three of the aldohexoses, *i.e.,* glucose, d(+)mannose and galactose, supported growth and streptomycin production though the yields were markedly different depending upon the nitrogen source used in the medium. In the $(NH_4)_2HPO_4$ and casein-digest mediums, glucose was the best of the three sugars, with galactose intermediate and d(+)mannose supporting the lowest yields. However, in the medium containing 1(—)proline as the sole nitrogen source, the use of d(+)mannose resulted in exceptionally high streptomycin broth potencies. High yields were also obtained with glucose in combination with 1(—)proline but not with galactose plus 1(—)proline.

Two ketohexoses were tested. Sorbose would not support growth in the $(NH_4)_2HPO_4$ medium. In addition, when sorbose was added to the casein-digest medium, lower yields were obtained than in the casein-digest medium to which no sugar had been added. Also, no growth was obtained in the 1(—)proline me-

dium to which sorbose was added. Levulose, on the other hand, was a relatively good carbohydrate, though not so satisfactory as glucose.

In all, five disaccharides were tested. All of the C_4-disaccharides, *i.e.*, cellobiose, lactose, and maltose, supported growth and streptomycin production in the $(NH_4)_2HPO_4$ medium. Of these three, maltose was superior when used in conjunction with an inorganic nitrogen source. In the casein-digest medium, however, approximately comparable yields were obtained with all three. When $1(-)$proline served as the sole nitrogen source, the use of maltose resulted in yields far superior to those obtained through the use of lactose or cellobiose.

Melibiose, a C_6-disaccharide, supported the best yields when used in the medium with $1(-)$proline. When used with the inorganic nitrogen compound, no growth was obtained.

Sucrose, a disaccharide with the hexose molecules linked through the reducing groups, was a poor carbon source in the $(NH_4)_2HPO_4$ medium. In addition, sucrose was also an inferior carbohydrate in the casein-digest and $1(-)$proline mediums.

The two trisaccharides, namely, melezitose and raffinose, do not offer much promise. Growth was not obtained when they were used in the $(NH_4)_2HPO_4$ medium, and low broth potencies were produced when they were used in conjunction with the organic nitrogen sources.

Of the polysaccharides, only inulin failed to support growth when used with an inorganic nitrogen compound. In addition, low streptomycin broth potencies were obtained in the inulin-casein digest and inulin-$1(-)$proline mediums. Dextrin and starch, however, appear to be promising carbohydrates, though neither of the lots tested was as good as glucose in the casein-digest or $(NH_4)_2HPO_4$ mediums. Both dextrin and starch supported relatively high yields in the $1(-)$proline mediums. The results would seem to warrant the testing of a series of dextrins and starches.

Alcohols: In all, eleven alcohols were tested in the three mediums. Of these eleven, only glycerol and mannitol supported growth in the $(NH_4)_2HPO_4$ medium. The use of glycerol resulted in relatively low streptomycin broth potencies in all three

mediums. Mannitol, however, when used in the $(NH_4)_2HPO_4$ or $1(-)$proline mediums, supported yields about equal to those obtained with the use of glucose in these mediums.

The addition of the other alcohols to the casein digest or $1(-)$proline mediums resulted in lower yields than were obtained in these two mediums to which no alcohol was added. Sorbitol exerted the most deleterious effect. No streptomycin was formed in the casein digest of $1(-)$proline mediums to which sorbitol was added.

Organic Acids: The fifteen organic acids listed in table III were tested in the $(NH_4)_2HPO_4$ and casein-digest mediums. The $1(-)$proline medium was not used.

When the organic acids were added to the casein-digest medium, the resulting streptomycin broth potencies were always lower than those obtained in the casein-digest medium to which no organic acid was added. Only seven of these acids supported growth and streptomycin formation in the medium with inorganic nitrogen. The highest broth potency obtained was 22 γ/ml. in the pyruvic acid $(NH_4)_2HPO_4$ medium.

The results with organic acids indicate that they offer little promise when used singly in place of carbohydrates in synthetic streptomycin fermentation mediums.

It is obvious from the above results that quite high streptomycin broth potencies may be obtained in synthetic mediums. Moreover, the specific carbon source added to these mediums greatly affects the fermentation.

Among the sugars, the pentoses and trisaccharides do not offer much promise when used as single carbohydrates. A number of the hexoses, disaccharides, and polysaccharides supported quite high streptomycin titers. There seems to be no clear correlation between the structure of the sugar molecule and the yield obtained. A number of these more promising sugars, however, are composed of glucose molecules.

With the exception of mannitol, none of the alcohols supported high yields. The organic acids that were tested were likewise poor carbon sources.

In analyzing the above results, however, several points should be borne in mind. The compounds tested were added singly to

the three mediums. With an increasing knowledge of the mechanism operating in the streptomycin fermentation, various combinations of these compounds might be used to advantage. In addition, these compounds were tested with one set of fermentation conditions using only three different nitrogen sources. The nitrogen source has a striking effect on the availability of the carbohydrate. The results obtained in the $(NH_4)_2HPO_4$ and $l(-)$proline mediums to which $d(+)$mannose was added serve as examples. Low streptomycin broth potencies were obtained in the mannose-$(NH_4)_2HPO_4$ medium, whereas good growth and high streptomycin broth potencies were obtained in the mannose-proline medium. In an attempt to stimulate streptomycin production in the mannose-$(NH_4)_2HPO_4$ medium, a number of organic acids, *i.e.,* acetic, citric, lactic, fumaric, pyruvic, succinic, and malic, were added as individual supplements at a 0.05 per cent level to this medium. In no instance did a stimulation of streptomycin production occur. Several amino acids, *i.e.,* dl-alpha-alanine, l-aspartic acid, $l(-)$proline, $l(-)$leucine, l-glutamic acid, and glycine, were also used as individual supplements to this medium. Again there was no stimulation of streptomycin production.

It should also be noted that the criterion used in these experiments was streptomycin broth potency and not growth. It does not necessarily follow that good growth will result in high streptomycin titers.

SUMMARY

1. A number of sugars, alcohols, and organic acids were tested as carbon sources in three streptomycin fermentation mediums.

2. The streptomycin broth potency obtained was greatly affected by the carbon source as well as by the source of nitrogen employed in the medium. The highest yields were obtained in the medium with $l(-)$proline as the sole nitrogen source.

3. The pentoses were poor carbon sources.

4. Among the hexoses, glucose and mannose supported the highest yields. The high streptomycin titers obtained by the use of mannose occurred only in the medium containing $l(-)$proline as the nitrogen source.

5. Maltose was the best of the disaccharides.

6. The trisaccharides were inferior carbohydrates under the experimental conditions that were used.

7. The only polysaccharide supporting low yields was inulin. The results obtained with the starch and dextrin would appear to warrant the testing of a series of these compounds.

8. Among the alcohols, only mannitol was a promising carbon source.

9. None of the organic acids supported high streptomycin broth potencies.

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THE ALBERT COMMONS * COLLECTION OF FUNGI IN THE HERBARIUM OF THE ACADEMY OF NATURAL SCIENCES IN PHILADELPHIA

DAVID R. SUMSTINE

This collection of fungi has been stored in the Academy of Natural Sciences for many years. The specimens are still in the original containers, such as newspapers, small match boxes, and other boxes, and identifications as well as other data are written on the blank parts of the newspaper and on the boxes. Some collections have a printed label. With the exception of the agarics, the specimens are well preserved for the most part and the material is generally ample. The specimens of agarics were pressed and only a few have been identified. There are no field notes giving the color, texture and other desirable characters for identification.

Most of the material was evidently identified by J. B. Ellis in whose herbarium duplicates of most of these specimens probably will be found. But many specimens were also sent to Charles H. Peck, A. B. Seymour, and William Trelease for identification. The name of the person identifying the specimen is generally given, and many are marked as new species. The original description usually refers definitely to the collection number and locality given on the label of the specimen. From this it would appear that these specimens are types or isotypes. Most of the specimens were collected in Delaware but a few came from New Jersey, Maryland, and Pennsylvania.

This collection has intrigued me for many years as I made frequent visits to the Academy of Natural Sciences. Some time ago,

* Albert Commons was born in Doe Run, Chester County, Pennsylvania, January 23, 1829. He became interested in Botany through his half-brother Franklin Commons. He began collecting in Delaware in 1842. His collections consist of flowering plants, mosses, hepatics, lichens and fungi. He died in 1919.

I suggested to Dr. F. W. Pennell, Curator of Plants, that the collection should be made available to mycologists for study, and offered to undertake its arrangement if the Academy would provide the necessary supplies. My offer was readily accepted and the specimens have been placed in suitable packets with printed labels.

No attempt has been made to make a critical study of these more than 4,000 specimens. The purpose is only to arrange them and make them accessible for study. Mycologists will find here a rich field for investigation.

In presenting a list of the species in one group, the Hyphomycetes, I hope to give a general idea of this collection. This enumeration will include the name of the species as given by the person identifying it, the collection number, the host, the locality, and the date.

A few personal notes will give some information that seemed valuable. When a specimen is marked as a new species, the original publication is given. This means that the publication refers directly to that specimen. Some of these specific names are, no doubt, synonyms and some should be transferred to other genera. This transfer will be left for specialists in the various groups. Probably at some future date, an enumeration of the other groups will be published.

All correspondence concerning permission to examine these specimens should be addressed to Dr. F. W. Pennell, Academy of Natural Sciences, Philadelphia, Pa.

HYPHOMYCETES

FAMILY MUCEDINACEAE. 1. *Botrytis fulva* Lk., (*1472*), on the ground; Wilmington, June 18, 1890. 2. *Botrytis geniculata* Cda., (*2026*), on *Carya;* Naaman's Creek, Nov. 14, 1892. 3. *Botrytis geniculata.* Cda., (*2278*), on *Hypoxylon;* Wilmington, Sept. 29, 1893. 4. *Botrytis geniculata* Cda., (*2221*), on *Hypoxylon;* Wilmington, Aug. 18, 1893. 5. *Botrytis streptothrix* (C. & E.) Sacc., (*2216*), (*Polyactis streptothrix* C. & E.), on *Orontium aquaticum;* Newark, Sept. 1, 1893. 6. *Botrytis streptothrix* (C. & E.) Sacc., (*1429*), on *Symplocarpus foetidus;* Wilmington,

May 18, 1890. *7. Botrytis streptothrix* (C. & E.) Sacc., *(1484)*, on *Hydrastis canadensis;* Wilmington, July 10, 1890. *8. Botrytis streptothrix* (C. & E.) Sacc., *(2081)*, on *Hydrastis canadensis;* Wilmington, June 29, 1893. *9. Botrytis streptothrix* (C. & E.) Sacc., *(2487)*, on *Arisaema triphyllum;* Naaman's Creek, July 6, 1894. *10. Botrytis vulgaris* Fr., *(1792)*, on *Staphylea trifolia;* Wilmington, July 7, 1891. *11. Oidium megalosporum* B. & C., *(2003)*, on decayed wood; Wilmington, Oct. 29, 1892. *12. Oidium simile* Berk., *(2745)*, *(Monilia aurea-fulva)*, on decayed wood; Wilmington, Oct. 15, 1890. *13. Oidium simile* Berk., *(1835)*, on decayed wood; Wilmington, Oct. 1, 1891. *14. Oidium simile* Berk., *(2288)*, on decayed wood; Wilmington, Aug. 18, 1890. *15. Oidium simile* Berk., *(1998)*, on decayed wood; Ogden Station, Sept. 15, 1892. *16. Piricularia grisea* (Cke.) Sacc., *(120)*, *(Tricothecium griseum* Cke.), on *Panicum sanguinale;* Faulkland, Aug. 21, 1885. Note: See number 42. *17. Piricularia grisea* (Cke.) Sacc., *(1539)*, var. *parasitica* E. & E. nov. var., in ed., on *Phyllachora graminis;* Wilmington, Aug. 18, 1890. *18. Polyscytalum sericeum* Sacc., *(552)*, on oak leaves; Faulkland, July 22, 1887.

19. Ramularia andromedae E. & M., *(1431)*, on *Andromeda ligustrina;* Wilmington, May 29, 1890. *20. Ramularia andromedae* E. & M., *(1481)*, on *Andromeda ligustrina;* Wilmington, June 19, 1899. *21. Ramularia arvensis* Sacc., *(71)*, on *Potentilla canadensis;* Faulkland, Sept. 12, 1885. *22. Ramularia arvensis* Sacc., *(49)*, No data. *23. Ramularia barbareae* Pk., *(1184)*, on *Barbarea vulgaris;* Wilmington, Dec. 19, 1889. *24. Ramularia celastri* E. & M., *(613)*, on *Celastrus scandens;* Faulkland, Aug. 10, 1887. *25. Ramularia heraclei* (Oud.) Sacc., *(1616)*, on *Heracleum lanatum;* Wilmington, Sept. 24, 1890. *26. Ramularia macrospora* Fres., *(= 216)*, on *Rumex obtusifolia;* Naaman's Creek, July 10, 1893. Note: This has been identified also as *R. decipiens* E. & E. and *R. obovata* (Fckl.) Sacc. *27. Ramularia orontii* E. & M., *(2215)*, on *Orontium aquaticum;* Newark, Sept. 8, 1893. *28. Ramularia plantaginis* E. & M., *(2186)*, on *Plantago major;* Wilmington, Aug. 7, 1893. *29. Ramularia pratensis* Sacc., *(216)*, on *Rumex obtusifolia;* Faulkland, Aug. 25, 1885. *30. Ramularia pratensis* Sacc., *(= 216)*, on *Rumex crispus;* Wilming-

ton, May 22, 1890. Note: The species on *Rumex crispus* seems to be generally labeled as *R. decipiens* E. & E., 31. *Ramularia pratensis* Sacc., (*612*), on Rhubarb; Delaware City, Aug. 12, 1887. 32. *Ramularia pratensis* Sacc., (= *216*), on *Rumex* sp.; Wilmington, Nov. 6, 1893. 33. *Ramularia ranunculi* Pk., (*1418*), on *Ranunculus* sp.; Newark, May 21, 1890. 34. *Ramularia Tulasnei* Sacc., (*50*), on *Fragaria virginiana;* Wilmington, Oct. 17, 1889. 35. *Rhinotrichum Curtisii* Berk., (*1581*), on decayed wood; Wilmington, July 21, 1890. 36. *Rhinotrichum Curtisii* Berk., (*2279*), on decayed wood; Wilmington, Sept. 28, 1893. 37. *Rhinotrichum ramosissimum* B. & C., (*1636*), on decayed wood; Sept. 30, 1890. 38. *Rhinotrichum ramosissimum* B. & C., (*1490*) and (*581*), on decayed wood; Wilmington, July 7, 1890. 39. *Sepedonium chrysospermum* (Bull.) Fr., on agaric; Mt. Cuba, July 19, 1894. 40. *Sporotrichum Bombacinum* Lk., (*1080*), on pine boards; Wilmington, Oct. 22, 1889. 41. *Trichoderma lignorum* (Tode) Harz., (*2194*), on *Polyporus;* Wilmington, Sept. 11, 1893. 42. *Trichothecium griseum* Cke., (*120*), on *Panicum sanguinale;* Faulkland, Aug. 21, 1885. Note: See number 16. 43. *Trichothecium roseum* (Pers.) Lk., (*2103*) and (*667*), on *Platanus occidentalis;* Naaman's Creek, July 10, 1893.

FAMILY DEMATIACEAE. 1. *Alternaria brassicae* Berk., (*94*), on cabbage; Faulkland, Oct. 12, 1885. 2. *Cercospora acalyphae* Pk., (*170*), on *Acalypha virginica;* Faulkland, Sept. 12, 1885. 3. *Cercospora acnidae* E. & E. n. sp., (*1011*), on *Acnida cannabia;* Wilmington, Sept. 1, 1889; Sept. 30, 1890, Proc. Acad. Phil. **43**: 89. 1891. Note: See also specimen *2582* in N.A.F. 4. *Cercospora ageratoides* E. & E., (*1842*), on *Eupatorium ageratoides;* Wilmington, Oct. 9, 1891. 5. *Cercospora ageratoides* E. & E., (*2599*), on *Eupatorium ageratoides;* Wilmington, Oct. 22, 1894. 6. *Cercospora althaeina* Sacc., (*103*), on *Abutilon avicennae;* Faulkland, Sept. 19, 1895. 7. *Cercospora althaeina* Sacc., (*183*), on *Malva rotundifolia;* Faulkland, Oct. 2, 1885. 8. *Cercospora ampelopsidis* Pk., (*51*), on *Ampelopsis quinquefolia;* Faulkland, July 10, 1885. 9. *Cercospora ampelopsidis* Pk., (*58*), on *Tecoma radicans;* Faulkland, Sept. 12, 1885. 10. *Cercospora asclepiadis* Ellis, (*846*), on *Acerates viridiflora;* Centerville, Aug. 11, 1875. Note: Near *Cercospora venturioides* Pk. 11. *Cercospora as-*

clepiadis Ellis, *(2468)*, on *Asclepias cornuti;* Stanton, July 4, 1894. 12. *Cercospora atra* E. & E. n. sp., *(591)*, on *Diospyrus virginiana;* Faulkland, Aug. 8, 1887, Jour. Myc. **4**: 4. 1888. 13. *Cercospora avicularis* Wint., on *Polygonum aviculare;* Faulkland, Aug. 1886. 14. *Cercospora boehmeriae* Pk., *(2214)*, on *Boehmeria cylindrica;* Wilmington, Sept. 5, 1893. 15. *Cercospora brachiata* E. & E. n. sp., *(626)*, on *Amaranthus retroflexus;* Faulkland, Aug. 18, 1887, Jour. Myc. **4**: 5. 1888. 16. *Cercospora brachiata* E. & E., *(2600)*, on *Amaranthus blitoides;* Wilmington, Oct. 22, 1894. 17. *Cercospora briareus* E. & E. n. sp., *(2537)*, on *Acerates viridifolia;* Elkton, Maryland, Aug. 21, 1894, Proc. Acad. Phil. **46**: 381. 1894. 18. *Cercospora callae* Pk. & Clint., *(524)*, on *Peltandra virginica;* Collins Beach, June 13, 1887. 19. *Cercospora cana* Sacc., *(334)*, on *Erigeron annuum;* Faulkland, Sept. 10, 1886. 20. *Cercospora cana* Sacc., *(2714)*, on *Erigeron annuum;* Wilmington, July 2, 1895. 21. *Cercospora cana* Sacc., on *Erigeron* sp.; Newark, July 7, 1893 (= *Cercosporella cana* = *Ramularia cana* = *Fusidium cana*).

22. *Cercospora cephalanthi* E. & K., *(678)*, on *Cephalanthus occidentalis;* Faulkland, Oct. 3, 1887, Jour. Myc. **4**: 5. 1888. 23. *Cercospora cephalanthi* E. & K., *(190)*, on *Cephalanthus occidentalis;* Salem City, N. J., Sept. 1, 1885. 24. *Cercospora chenopodii* Fres., *(157)*, on *Chenopodium album;* Faulkland, Aug. 26, 1885. 25. *Cercospora chenopodii* Fres., *(157)*, on *Chenopodium album;* Faulkland, Sept. 10, 1885. 26. *Cercospora chenopodii* Fres., *(157)*, on *Chenopodium album;* Stanton, Sept. 10, 1885. 27. *Cercospora chenopodii* Fres., *(304)*, on *Chenopodium urbicum;* Faulkland, Aug. 16, 1886. 28. *Cercospora circumscissa* Sacc., *(148)*, on *Prunus cerasus;* Faulkland, Sept. 9, 1885. 29. *Cercospora clavata* (Ger.) Pk., *(651)*, on *Asclepias incarnata;* Wilmington, Oct. 22, 1889. 30. *Cercospora clavata* (Ger.) Pk., *(651)*, on *Asclepias cornuti;* Faulkland, Aug. 25, 1887. 31. *Cercospora condensata* E. & K., *(130)*, on *Gleditsia triacanthos;* Stanton, Sept. 10, 1885. 32. *Cercospora cruciferarum* E. & E. n. sp., *(249)*, on *Sisymbrium officinale;* Faulkland, June 16, 1887, Jour. Myc. **3**: 17. 1887. Note: Also collected at same place, Aug. 1, 1886. 33. *Cercospora daturae* Pk., *(668)*, on *Datura Stramonium;* Faulkland, Sept. 22, 1887.

34. *Cercospora demetriana* Wint., (*272*), on *Crotalaria sagittalis;* Faulkland, Aug. 2, 1886. 35. *Cercospora desmodii* E. & K., on *Desmodium viridiflorum;* Centerville, Sept. 16, 1865. 36. *Cercospora deutziae* E. & E. n. sp., (*199*), on *Deutzia gracilis;* Faulkland, Sept. 9, 1885, Jour. Myc. 4: 5. 1888. Note: Also collected at same place, Nov. 4, 1885. 37. *Cercospora diantherae* E. & K., (*2528*), on *Dianthera americana;* Delaware City, Aug. 17, 1894. 38. *Cercospora diodiae* Cke., (*273*), on *Diodia teres;* Faulkland, Aug. 1886. 39. *Cercospora dioscoreae* E. & M., (*373*), on *Dioscorea villosa;* Mt. Cuba, Aug. 19, 1886. Note: Two other specimens, one from Faulkland and one from Wilmington. 40. *Cercospora echinocystis* E. & M., (*171*), on *Sicyos angustatus;* Faulkland, Sept. 10, 1885. 41. *Cercospora effusa* B. & C., (*2315*), on *Lobelia syphilitica;* Wilmington, Oct. 9, 1893.

42. *Cercospora elephantopidis* E. & E. n. sp., (*75*) and (*218*), on *Elephantopus carolinianus;* Faulkland, Sept. 27, 1885, Jour. Myc. 3: 15. 1887. Note: The original spelling is *elephantopi.* 43. *Cercospora ferruginea* Fckl., (*1619*), on *Lobelia puberula;* Wilmington, Sept. 28, 1890. 44. *Cercospora ferruginea* Fckl. (*132*), on *Ambrosia trifida;* Faulkland, Sept. 8, 1885. Note: There are two more unnumbered specimens from same place. 45. *Cercospora flagellaris* E. & M., (*237*), on *Phytolacca decandra;* Faulkland, Sept. 27, 1885. Note: Another specimen (*624*) from the same place. 46. *Cercospora galii* E. & Holw., (*290*), on *Galium pilosum;* Faulkland, Aug. 13, 1886. 47. *Cercospora gentianicola* E. & E. n. sp., (*128*), on *Gentiana crinita;* Faulkland, Oct. 17, 1887, Jour. Myc. 4: 2. 1888. 48. *Cercospora gnaphaliaceae* Cke., (*2444*), on *Gnaphalium purpureum;* Smyrna, June 18, 1894. Note: Also specimen from Faulkland, number *627.* 49. *Cercospora granuliformis* E. & Holw., (*33*), on *Viola cucullata;* Faulkland, July 16, 1885. 50. *Cercospora heucherae* E. & M., (*322*), on *Heuchera americana;* Wilmington, Oct. 22, 1889. 51. *Cercospora heucherae* E. & M., (*1205*), on *Heuchera americana;* Wilmington, Feb. 5, 1890. 52. *Cercospora houstoniae* E. & E. n. sp., (*1371*), on *Houstonia coerulea;* Wilmington, Apr. 28, 1890, Proc. Acad. Phila. 43: 89. 1891. 53. *Cercospora hydrocotylis* E. & E., (*2568*), on *Hydrocotyle umbellata;* Pennsville, N. J., Sept. 17, 1894. 54. *Cercospora infuscans* E. & E. n

sp., *(1621)*, on *Rhus venenata;* Porters Station, Oct. 9, 1890, Proc. Acad. Phila. **43**: 90. 1891. 55. *Cercospora infuscans* E. & E., *(1621)*, on *Rhus venenata;* Newark, Oct. 10, 1893. 56. *Cercospora leptosperma* Pk., *(324)*, on *Aralia nudicaulis;* Mt. Cuba, Aug. 19, 1886. 57. *Cercospora lini* E. & E. n. sp., *(248)*, on *Linum virginianum;* Faulkland, Sept. 1886, Jour. Myc. **3**: 16. 1887. Note: Also two other specimens. 58. *Cercospora liquidambaris* E. & E., *(1005)*, on *Liquidambar styracifolia;* Wilmington, Sept. 20, 1889. 59. *Cercospora monoica* E. & Holw., *(32)*, on *Amphicarpa monoica;* Faulkland, July 13, 1885. 60. *Cercospora nesaeae* E. & E. n. sp., *(1984)*, on *Nesaea verticillata;* Milford, Sept. 1, 1892, Proc. Acad. Phila. **45**: 170. 1893. 61. *Cercospora nesaeae* E. & E., *(= 1984)*, on *Nesaea verticillata;* Delaware City, Aug. 17, 1894. 62. *Cercospora nymphaeaceae* C. & E., *(2730)*, on *Nymphaea advena;* Rehoboth City, Aug. 1, 1895. 63. *Cercospora nymphaeaceae* C. & E., *(300)*, on *Nymphaea advena;* Wilmington, Sept. and Oct. 1894. 64. *Cercospora oculata* E. & K., *(109)*, on *Verononia noveboracensis;* Faulkland, Sept. 8, 1885. 65. *Cercospora oculata* E. & K., *(139)*, on *Vernonia noveboracensis;* Faulkland, Aug. 15, 1885. 66. *Cercospora osmorrhizae* E. & E. n. sp., *(1416)*, on *Osmorrhiza longistylis;* Wilmington, May 29, 1890, Proc. Acad. Phila. **43**: 89. 1891. 67. *Cercospora pachyspora* E. & E. n. sp., *(1013* and *1014)*, on *Alisma plantago;* Wilmington, Oct. 4, 1889, Proc. Acad. Phila. **43**: 88. 1891. 68. *Cercospora polygonacea* E. & E., *(159)*, on *Polygonum dumetorum;* Faulkland, Aug. 25, 1885. 69. *Cercospora polygonacea* E. & E., *(167)*, var. *avicularis,* on *Polygonum aviculare;* Faulkland, Aug. 25, 1885.

70. *Cercospora polygonorum* Cke., *(169)*, on *Polygonum sagittatum;* Faulkland, Sept. 12, 1885. 71. *Cercospora polygonorum* Cke., *(118)*, on *Polygonum aviculare;* Faulkland, Aug. 15, 1885. 72. *Cercospora polygonorum* Cke., *(168)* and *(162)*, on *Polygonum persicaria;* Faulkland, Sept. 12, 1885. Note: This may be *Cercospora hydropiperis* (Thum.) Speg. 73. *Cercospora racemosa* E. & M., *(277)*, on *Teucrium canadense;* Faulkland, Oct. 12, 1885. 74. *Cercospora rhuina* C. & E., *(86)*, *(57)* and *(56)*, on *Rhus glabra;* Faulkland, Aug. 6, 1885. 75. *Cercospora rhuina* C. & E., on *Rhus glabra;* Wilmington, July 3, 1894. 76. *Cerco-*

spora sabbatiae E. & E. n. sp., *(589)*, on *Sabbatia angularis;* Faulkland, Aug. 2, 1887, Jour. Myc. **4**: 3. 1888.   77. *Cercospora sagittariae* E. & K., *(221)*, on *Sagittaria variabilis;* Faulkland, Aug. 2, 1886.   78. *Cercospora sagittariae* E. & K., *(321)*, on *Sagittaria variabilis;* Wilmington, Sept. 4, 1889.   79. *Cercospora sagittariae* E. & K., *(2572)*, on *Sagittaria variabilis;* Delaware City, Sept. 18, 1894.   80. *Cercospora sanguinariae* Pk., *(2746)* and *(6)*, on *Sanguinaria canadensis;* Faulkland, June 12, 1885. 81. *Cercospora senecionis* E. & E. n. sp., *(978)*, on *Senecio aureus;* Wilmington, Nov. 1, 1889, Proc. Acad. Phila. **43**: 90. 1891.   82. *Cercospora serpentaria* E. & E. n. sp., *(337)*, on *Aristolochia serpentaria;* Faulkland, Sept. 11, 1886, Jour. Myc. **3**: 13. 1887. 83. *Cercospora simulata* E. & E., *(381)*, on *Cassia Marylandica;* Faulkland, Sept. 26, 1886.   84. *Cercospora smilacina* Sacc., on *Smilax glauca;* Sept. 7, 1892.   85. *Cercospora smilacis* Thum., *(63)*, on *Smilax glauca;* Faulkland, July 23, 1885.   86. *Cercospora smilacis* Thum., *(761)*, on *Smilax rotundifolia;* Faulkland, Nov. 18, 1887.   87. *Cercospora squalidula* Pk., *(461)*, on *Clematis virginiana;* Faulkland, Oct. 8, 1886.   88. *Cercospora Stylosanthes* E. & E. n. sp., *(336)*, on *Stylosanthes elatior;* Faulkland, Sept. 17, 1886, Jour. Myc. **3**: 13. 1887.   Note: Possibly the same as *Cercospora Commonsii.* 89. *Cercospora symplocarpi* Pk., *(98)*, on *Symplocarpus foetidus;* Faulkland, Aug. 15, 1885.   Note: Three other collections from different places.   90. *Cercospora thaspii* E. & E. n. sp., *(1034)*, on *Thaspium trifoliatum;* Wilmington, Oct. 23, 1889.   Note: Found no publication.   91. *Cercospora tuberosa* E. & K., *(673)*, on *Apios tuberosa;* Faulkland, Sept. 30, 1875.   92. *Cercospora verbasicola* E. & E. n. sp., *(669)*, on *Verbascum thapsus;* Faulkland, Aug. 26, 1887, Jour. Myc. **4**: 3. 1888.   93. *Cercospora violae* Sacc., *(236)*, on *Viola cucullata;* Faulkland, Sept. 29, 1885.   94. *Cercospora* sp., *(672)*, on *Rhyncosia tomentosa;* Aug. 5, 1874.   95. *Cladosporium carpophyllum* Thum., *(= 321)*, on peaches; Wilmington, Sept. 1889.   96. *Cladosporium dendriticum* Wallr., *(12)*, on apple; Faulkland, Jan. and Feb. 1886.   97. *Cladosporium epimyces* Cke., *(1879)*, on *Polyporus;* Wilmington, Oct. 26, 1891.

    98. *Cladosporium epimyces* Cke., *(480)*, on *Agaricus;* Faulkland, March 9, 1887.   99. *Cladosporium epiphyllum* Pk., *(1379)*,

on leaf; Wilmington, April 28, 1890. 100. *Cladosporium fulvum* Cke., (*2378*), on *Lycopersicum;* Wilmington, Nov. 28, 1883. 101. *Cladosporium graminum* Cke., (*2060*), on *Carex torta;* Naaman's Creek, July 10, 1893. 102. *Cladosporium graminum* Cke., (*30*), on *Carex* sp.; Faulkland, July 22, 1885. 103. *Cladosporium herbarum* (Pers.) Lk., (*1772*), on *Asclepias cornuti;* Wilmington, May 5, 1891. 104. *Cladosporium typharum* Desm., (*2208*), on *Typha latifolia;* Wilmington, Aug. 25, 1893. 105. *Cladosporium* 'sp., (*91*), on *Barbarea vulgaris;* Greenbank, July 17, 1886. 106. *Clasterosporium caespitulans* E. & E., on rotten wood; Newfields, N. J., 1879. 107. *Clasterosporium Commonsii* E. & E. n. sp., (*127*), on dead bark; Wilmington, Dec. 12, 1889. Note: Found no publication. 108. *Clasterosporium populi* E. & E. n. sp., (*1806*), on *Populus grandidentata;* Carrcroft, Aug. 16, 1891, Jour. Myc. '7: 134. 1894.

109. *Dendrina diospyri* B. & C., (*1585*), on *Diospyrus virginiana;* Wilmington, Sept. 18, 1890. Note: Possibly a *Trichosporium*. 110. *Fusella polyporina* E. & E. n. sp., (*2728*), on *Fomes lucidus;* Selbyville, July 18, 1895. Note: Found no publication. 111. *Fusicladium dendriticum* (Wallr.) Fckl., (*154*) and (*55*), on apple; Faulkland, Aug. 25, 1885. 112. *Fusicladium staticis* E. & E. n. sp., (*2565*), on *Statice limonum;* Cape May, N. J., Sept. 13, 1894, Proc. Acad. Phila. **46**: 378. 1894. 113. *Glenospora Curtisii* Berk., (*494*), on *Nyssa multiflora;* Faulkland, May, 1887. 114. *Glenospora Curtisii* Berk., on *Nyssa multiflora;* Wilmington, Dec. 19, 1889. 115. *Glenospora Curtisii* Berk., on *Nyssa multiflora;* Faulkland, Feb. 1887. Note: Possibly *Dematium ramorum*. 116. *Gymnosporium arundinis* Cda., (*1816*), on *Panicum* sp. Note: Possibly *Coniosporium arundinis* (Cda.) Sacc. 117. *Helminthosporium arctisporum* C. & E., (*1447*), on *Vaccinium corymbosum;* Wilmington, May 29, 1890. 118. *Helminthosporium hadotrichoides* E. & E. n. sp., (*347*), on *Eragrostis major;* Sept. 15, 1887, Jour. Myc. **4**: 44. 1888. 119. *Helminthosporium interseminatum* B. & R., (*1134*), on *Phytolacca decandra;* Wilmington, Jan. 1, 1890. 120. *Helminthosporium macrocarpum* Grev., (*1249*), on *Liriodendron tulipiferae;* Wilmington, Feb. 18, 1890. 121. *Helminthosporium macrocarpum* Grev., (*1384*), on *Magnolia glauca;* Wilmington, Feb. 3, 1890.

122. *Helminthosporium persistens* C. & E., *(2018)*, on oak bark; Naaman's Creek, Nov. 14, 1892. 123. *Helminthosporium pirorum* Lb., on apple; Faulkland, Aug. 1887. 124. *Helminthosporium tiliae* Fr., *(2124)*, on *Tilia americana;* Mt. Cuba, June 28, 1893. 125. *Helminthosporium variegatum* E. & E., *(1243)*, on oak sapling; Wilmington, Feb. 18, 1890. 126. *Helminthosporium* sp., *(2086)*, on *Salix humilis;* Newark, July 7, 1893. 127. *Helmintho-sporium* sp., *(60)*, on *Solidago lanceolata;* Faulkland, Oct. 11, 1885. Note: "I have seen this before but I think it has never been named." J. B. E.

128. *Macrosporium avicennae* E. & E. n. sp., *(1586)*, on *Abutilon avicennae;* Wilmington, Sept. 9, 1890. Note: Found no publication. 129. *Macrosporium commune* Rab., *(1936)*, on *Dianthus* sp.; Wilmington, Apr. 26, 1892. 130. *Macrosporium cydoniae* E. & E. n. sp., *(2445)*, on quince; Pensauken, June 12, 1894. Note: Found no publication. 131. *Macrosporium herculeum* E. & M., *(243)*, on *Nasturtium armoracea;* Pennsgrove, N. J., July 13, 1886. 132. *Macrosporium iridis* E. & M., *(1230)*, on *Iris* sp.; Wilmington, Dec. 24, 1889. 133. *Macrosporium maculatum* C. & E., *(110)*, on *Zea mays;* Faulkland, Aug. 21, 1885. 134. *Macrosporium maydis,* *(110* and *210)*, on *Zea mays;* Faulkland, Aug. 20 and 21, 1885. 135. *Macrosporium nobile* Vize., *(2385)*, on *Dianthus* sp.; Wilmington, Nov. 28, 1893. 136. *Macrosporium* sp., *(630)*, on *Asclepias cornuti;* Faulkland, Aug. 18, 1887. 137. *Macrosporium* sp., *(2421)*, on *Trifolium incarnatum;* Pleasant Hill, May 28, 1894. 138. *Macrosporium* sp., *(680)*, on *Maclura aurantiaca;* Faulkland, Oct. 3, 1887. Note: Also *Phoma* on this host. 139. *Macrosporium* sp., *(1778)*, on *Ailanthus glandulosa;* Wilmington, Apr. 1, 1891. 140. *Macrosporium* sp., *(200)*, on?; Faulkland, Sept. 9, 1885. Note: "I do not like to make any more species." J. B. E. 141. *Macrosporium* sp., *(2035)*, on *Sarracenia purpurea;* Laurel, June 15, 1893. 142. *Macrosporium* sp., *(630)*, on *Asclepias;* Aug. 18, 1886. Note: Same as number *136*. 143. *Periconia byssoides* Pers., *(346)*, on *Commelina nudiflora;* Faulkland, Sept. 17, 1886. Note: Possibly same as *Sporocybe byssoides* Fr. 144. *Periconia minituissima* Cda., *(938)*, on *Morus alba;* Wilmington, Aug. 2, 1889. 145. *Periconia pycnospora* Fres., *(2138)*, on *Impatiens fulva;*

Wilmington, July 27, 1893. Note: Also numbers *2222, 2255, 2462, 2361, 2246, 1224.* 146. *Polythrincium trifolii* Kunze., (*35 and 318*), on *Trifolium repens;* Faulkland, Sept. 29, 1885. 147. *Scolecotrichum graminis* Fckl., (*519*), on *Dactylis glomerata;* Faulkland, June 12, 1887. 148. *Scolecotrichum graminis* Fckl., (*1886*), on *Muhlenbergia mexicana;* Pennsville, N. J., Oct. 15, 1891. 149. *Scolecotrichum graminis* Fckl., (*532*), on *Phleum pratense;* Faulkland, July 18, 1887. 150. *Septonema spilomeum* Berk., (*392* and *554*), on old boards; Faulkland, Sept. 21, 1886. 151. *Septonema spilomeum* Berk., (*944*), on *Morus alba;* Wilmington, July 22, 1889. 152. *Septosporium fuliginosum* Ell., (*1745*), on *Cornus florida;* Wilmington, May 5, 1890. 153. *Septosporium hadotrichoides* E. & E. n. sp., (*347*), on *Eragrostis major;* Faulkland, Sept. 17, 1886. Note: Jour. Myc. **4**: 44. 1888. Published as *Helminthosporium hadotrichoides.* 154. *Sporodesmium antiquum* Cda., (*985*), on *Platanus occidentalis;* Wilmington, 1889, 1891. 155. *Sporodesmium concinnum* Berk., (*2020*), on old wood; Carrcroft, Dec. 4, 1892. Note: Also numbers *2252, 1741.* 156. *Sporodesmium conglobatum* C. & E., (*1652*), on *Quercus palustris;* Wilmington, Oct. 31, 1890. 157. *Sporodesmium hysterroideum* C. & E., (*1995*), on *Juniperus virginiana;* Wilmington, Oct. 29, 1892. 158. *Sporodesmium lepraria* B. & Br., (*2021*), on decayed wood; Carrcroft, Dec. 4, 1892. 159. *Sporodesmium peziza* C. & E., (*1212*), on *Cornus florida;* Wilmington, Feb. 5, 1890. 160. *Sporodesmium peziza* C. & E., (*2081*), on *Tilia* sp.; Mt. Cuba, June 22, 1893. 161. *Streptothrix atra* B. & C., (*1201*), on *Vaccinium corymbosum;* Wilmington, Jan. 2, 1890. 162. *Streptothrix atra* B. & C., (*1905*), on *Juniperus virginiana;* Wilmington, Dec. 5, 1891. 163. *Torula binalis* C. & E., (*731*), on willow; Faulkland, Oct. 17, 1887. 164. *Torula Fuckelii* Sacc., (*1192*), on *Salix fragilis;* Wilmington, Feb. 5, 1890. 165. *Torula herbarum* Lk., (*2139*), on *Impatiens fulva;* Mt. Cuba, July 27, 1893. 166. *Zygodesmus bicolor* C. & E., (*2454*), on *Clavaria* sp.; Wilmington, June 23, 1894. 167. *Zygodesmus fuscus* Cda., (*716*), on bark; Faulkland, Oct. 15, 1887.

FAMILY STILBACEAE. 1. *Podosporium rigidum* Schw., (*2339*), on *Rhus toxicodendron;* Wilmington, Oct. 7, 1893. 2. *Stilbum aurifilum* Ger., (*2666*), on *Corticium* (*Stereum*) sp., Wilmington,

Nov. 15, 1894. 3. *Stilbum erythrocephalum* Detm. *(1470)*, on animal dung; Newark, June 27, 1890. 4. *Stilbum parvulum* C. & E., *(2286)*, on *Acer rubrum;* Wilmington, Sept. 30, 1893. 5. *Stilbum rhoidis* B. & C., *(1099)*, on *Rhus glabra;* Wilmington, Dec. 3, 1889. 6. *Stilbum rhoidis* B. & C., *(1403)*, on *Diospyros virginiana;* Wilmington, Jan. 28, 1890. 7. *Stilbum tomentosum* Schrad., *(1364)*, on moss; Wilmington, April 2, 1890.

FAMILY TUBERCULARIACEAE. 1. *Aegerita candida* Pers., *(791)*, on leaves; Faulkland, Dec. 8, 1887. 2. *Dendrodochium affinis* Sacc., *(953)*, on *Euphorbia* sp.; Wilmington, Aug. 13, 1887. Note: Mixed with *Vermicularia compacta* C. & E. 3. *Dendrodochium densipes* Sacc. & Ell., *(1595)*, on *Salix;* Wilmington, Aug. 19, 1890, Jour. Myc. 4: 117. 1888. Note: Original description based on Commons collection. No. *639*. 4. *Dendrodochium rubellum* Sacc., *(2182)*, on *Platanus occidentalis;* Naaman's Creek, July 28, 1893. 5. *Dendrodochium pezizoides* E. & E. n. sp., *(2234)*, on *Triticum vulgare;* Newark, Sept. 8, 1893. Note: Found no publication. 6. *Dendrodochium serratum* E. & E. n. sp., *(739)*, on cedar bark; Faulkland, Oct. 24, 1887. Note: Found no publication. 7. *Dendrodochium succineum* E. & E. n. sp., *(1914)*, on decayed wood; Laurel, Feb. 24, 1892. Note: Found no publication. 8. *Epicoccum negundinis* E. & E. n. sp., *(2232)*, on *Negundo aceroides;* Grenogue, Aug. 18, 1893. Note: Found no publication. *Epicoccum negundinis* Otth. in Bern. Mittheil. 1879, seems to antedate Ellis' name. 9. *Epicoccum neglectum* Desm., *(1075)*, on *Panicum crus-galli;* Wilmington, Nov. 5, 1889. 10. *Epicoccum neglectum* Desm., *(2362)*, on *Scirpus lacustris;* Wilmington, Nov. 1, 1893. Note: Also seven other numbers. 11. *Epicoccum nigrum* Lk., *(2375)*, on *Typha latifolia;* Wilmington, Nov. 6, 1893. 12. *Epicoccum purpurascens* Ehrbg., *(952)*, on *Scirpus lacustris;* Wilmington, Aug. 13, 1889. 13. *Epicoccum purpurascens* Ehrbg., *(1910)*, on *Lycogala epidendrum;* Wilmington, Nov. 24, 1891. 14. *Epicoccum purpurascens* Ehrbg., *(211)*, on *Zea mays;* Faulkland, Sept. 8, 1885. 15. *Epicoccum scabrum* Cda., *(2582)*, on wood; Wilmington, Sept. 25, 1894. 16: *Epicoccum vulgare* Cda., *(1220)*, on *Euonymus americana;* Wilmington, Mar. 24, 1890. 17. *Epicoccum vulgare* Cda., *(1231)*, on *Iris;* Wilmington, Dec. 24, 1889. Note: Also number

2385. 18. *Fusarium aurantiacum* (Lk.) Sacc., *(1468)*, on *Zyga-denus helmanthoides;* Newark, June 27, 1890. 19. *Fusarium episphaericum* (C. & E.) Sacc., *(2245)*, on *Carya;* Wilmington, Sept. 2, 1893. Note: Originally described as *Fusisporium epi-sphaericum.* With this specimen is associated *Nectria bicolor* E. & E. n. sp., Proc. Acad. Phila. **45**: 443. 1893. The *Fusarium* is the conidial stage. 20. *Fusarium granulosum* E. & E. n. sp., *(2091)*, on *Smilax hispida;* Mt. Cuba, June 28, 1893, Proc. Acad. Phila. **45**: 466. 1893. 21. *Fusarium rimosum* (Pk.) Sacc., *(1623)*, on corn stalk; Wilmington, Oct. 11, 1890. Note: Originally de-scribed as *Fusisporium rimosum.* 22. *Fusarium roseum* Lk., *(1138)*, on *Phytolacca decandra;* Wilmington, Jan. 27, 1890. Note: Also numbers *1062* and *973.* 23. *Fusarium Schweinitzii* Ell. & Hark., *(1311)*, on *Vitis* sp.; Wilmington, Jan. 18, 1890. 24. *Fusarium* sp., *(2591)*, on beans; Wilmington, Oct. 2, 1894. 25. *Hymenopsis trochiloides* Sacc., *(2316)*, on *Scirpus lacustris;* Wilmington, Oct. 9, 1893. Note: Also numbers *2317, 2209, 2757, 2210.* 26. *Illosporium pallidum* Cke., *(1287)*, on leaves; Wilming-ton, Dec. 19, 1889. 27. *Illosporium panduratum* E. & E. n. sp., *(2277)*, on *Boletinus porosus;* Wilmington, Sept. 3, 1893. Note: Found no publication. 28. *Myrothecium inundatum* Sacc., *(601)*, on the ground; Faulkland, Aug. 8, 1887. 29. *Myrothecium inun-datum* Sacc., *(1817)*, on agaric; Wilmington, Sept. 7, 1891. 30. *Sphaerosporium lignatile* Schw., on *Fraxinus americana;* Wilming-ton, Oct. 18, 1890. Note: Considered a doubtful genus. 31. *Trimmatostroma americana* Thum., *(1690)*, on *Salix;* Wilming-ton, Nov. 1, 1890. 32. *Tubercularia menispermii* Schw., *(395)* and *(345)*, on *Menispermum canadense;* Faulkland, Oct. 6, 1886. 33. *Tubercularia nigricans* (Bull.) Lk., *(508)*, on *Fraxinus americana;* Faulkland, Mar. 20, 1887. 34. *Tubercularia vulgaris* Tode., *(2265)*, on dead stem; Mt. Cuba, Sept. 20, 1893. Note: Many other collections.

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SPECIES OF SYNCHYTRIUM IN LOUISIANA. V. A NEW SPECIES ON SAMBUCUS CANADENSIS

MELVILLE T. COOK

(WITH 9 FIGURES)

This species was found on the young leaves of young plants that were not more than six inches in height and growing in wet, usually muddy, soil. Infected plants growing in a dry environment were infected before the subsidence of the water and drying of the soil. The galls were most abundant on the under surfaces of the leaves but were found to some extent on the petioles and rarely on the upper surfaces of the leaves (FIGS. 1, 2, 3). The infections started as small, green spots in which small, bright green, hemispherical galls are formed. In the later stages, these galls were large and usually conical with a pit or crater-like cavity in the center (FIG. 8). The infected cell was at the bottom of a crater. The mature gall rested almost or entirely on the surface of the leaf or petiole but in some cases the basal part was embedded in the host tissues (FIG. 8).

The infections were in young epidermal cells (FIG. 4) as in all other species studied by the writer. The infected cells grew rapidly. The galls were formed by a growth of the epidermal and mesophyll cells surrounding the infected epidermal cells. The cells composing the galls may or may not be elongated (FIGS. 7, 8). When the galls were partly submerged, the cells of the host tissues were more or less modified (FIG. 8). When the galls were numerous they caused a more or less pronounced deformity of the leaf or petiole (FIG. 3). In some few cases, the epidermal cells of a new gall became infected which resulted in a pronounced compound gall. In some cases a second zoöspore endeavored to penetrate the infected cell through the opening in the cone. If it failed to do so, it increased in size for a time and died (FIG. 9).

The fungus was very light lemon color at first, became yellow, orange, and brown with age and was visible at the bottom of the crater at a very early period in the development of the gall. It filled the host cell in a very short time (FIG. 4). In many cases the fungus died and turned black before maturity. After a time the infected cell grew more rapidly than the fungus, which did not completely fill the host cell (FIGS. 5, 6, 7). The fungus was granular with a well defined nucleus. The segmentation of the
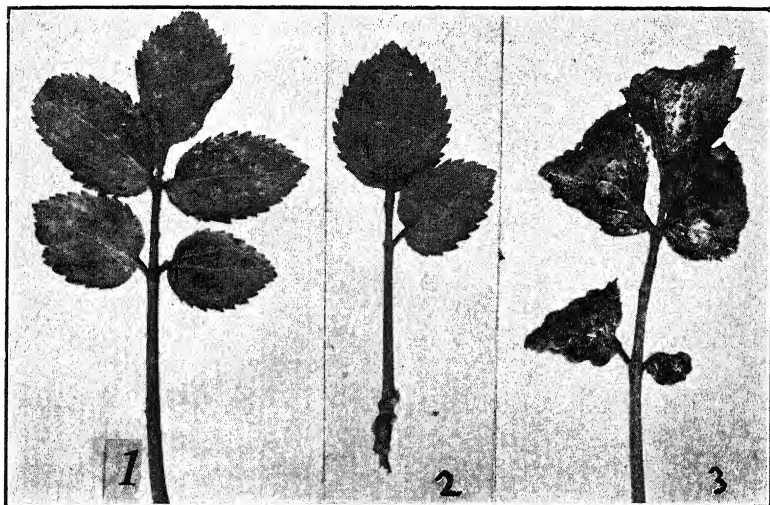
FIGS. 1-3. *Synchytrium sambuci.*

fungus to form sporangia was not observed. Many dead, empty galls were seen and the writer is of the opinion that the fungus became separated from the gall before the formation of the sporangia. The fungus grew rapidly and was surrounded by granular material or modified host cell contents which became modified into a dense granular or solid substance (FIGS. 5, 6). The infected cells were pear shaped (FIG. 7) or irregular (FIGS. 5, 6). In the latter case the contents of the cell appeared to be amoeboid but there was no evidence of disintegration of cell walls of the host plant. The host cell nucleus is very definite and occasionally two host nuclei are present. The host nuclei may or may not be attached to the nucleus. Occasionally two fungi were found in the
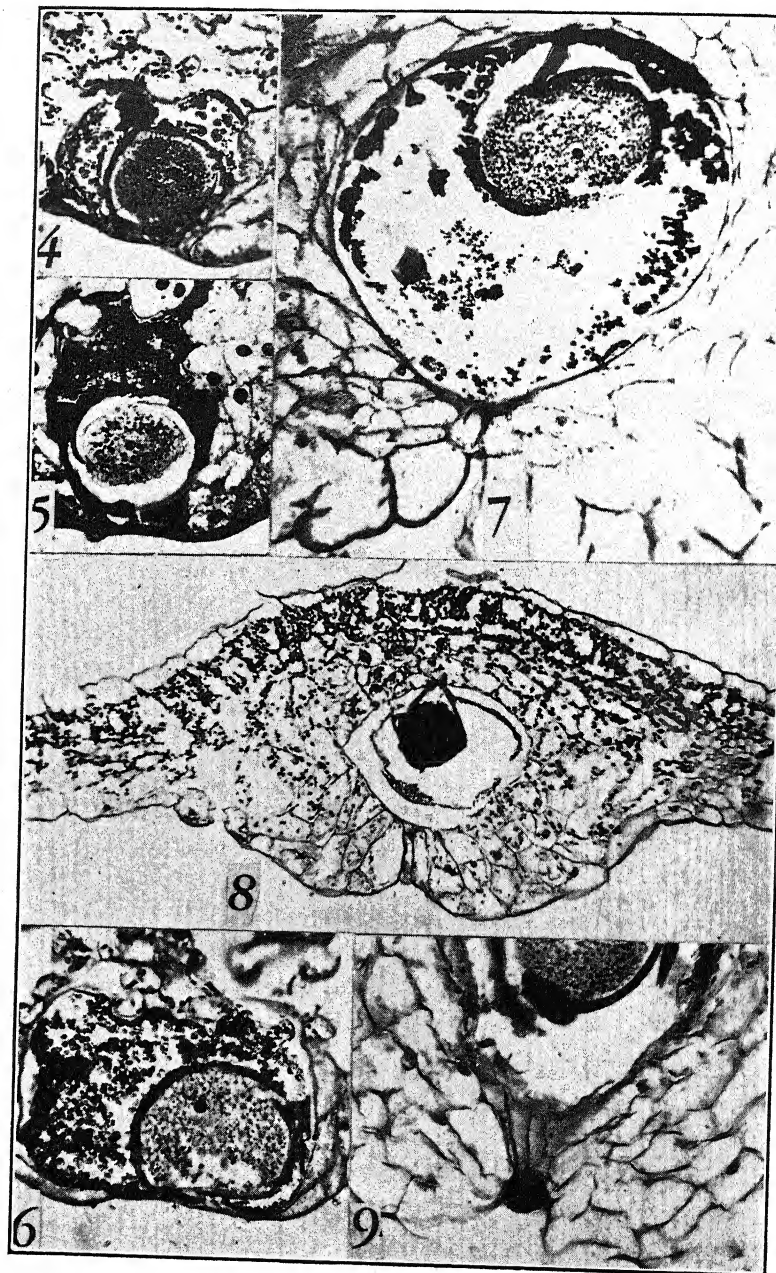
Figs. 4–9. *Synchytrium sambuci.*

same host cell. The fungus was usually surrounded by a hard wall which appeared to be composed of three layers, which break very easily (FIGS. 6, 7).

Synchytrium sambuci sp. nov.

Gallis numerosis in utraque foliorum superficie atque in petiolis; simplicibus, plerumque ampullaceis, superficie nixis vel in textu sitis, 75–100 μ diametro. Soris primo subflavissimis, aetate in colorem flavum, aurantiacum et brunneum se mutantibus, circa 135 μ diametro.

The author wishes to express his thanks to Dr. C. W. Edgerton for suggestions and for making the photographs, to Dr. P. J. Moorhead, who translated the description into Latin, and to Mr. W. J. Dickson, who found and collected the material.

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EXPLANATION OF FIGURES

FIGS. 1, 2. Single galls on leaves and petioles. 3. Leaf deformed by galls. 4. Infected epidermal cell showing fungus and host cell contents. 5. More advanced stage showing irregular host cell. 6. More advanced stage showing large host cell. 7, 8. More advanced stage showing opening to host cell. 7. A gall in which the tissues of the host are only slightly modified. 8. A gall in which the infected cell is partly submerged in the tissues of the leaf. 9. A gall showing a second enlarged spore trying to gain entrance.

OLPIDIOPSIS SCHENKIANA AND ITS HYPERPARASITE ECTROGELLA BESSEYI N. SP.

F. K. Sparrow and Bernard Ellison [1]

(WITH 1 FIGURE)

Late in November, 1945, a purple sediment was noticed on the bottom of an unfrozen pond (water temperature 6° C.) in the vicinity of Ann Arbor, Michigan. An examination of this material showed it to consist primarily of a colonial purple bacterium and occasional filaments of a one-banded, replicate-walled species of *Spirogyra*. Of particular interest was the fact that the alga was heavily parasitized by a phycomycetous fungus, readily recognizable as *Olpidiopsis Schenkiana* Zopf. Thalli, zoosporangia, and sex organs of the parasite were relatively plentiful in the algal filaments. A closer inspection of the material revealed a fact of unusual interest, namely, that the algal parasite was, in turn, parasitized by an endoparasite which, like its host, was also a phycomycete.

Olpidiopsis Schenkiana is well known from the classic researches of Zopf (13), and since his original discovery of the fungus it has been collected a number of times in Europe (2, 3, 4, 6, 8, 11, 12), once in India (1), and has been mentioned by Karling (5) as occurring in the vicinity of New York. Because of paucity of information concerning the species in the Western Hemisphere and because the presence of an endoparasite—apparently a new species—serves to offer an explanation of certain puzzling figures which have appeared in the European literature of this fungus, its occurrence in Michigan has seemed worthy of note.

OLPIDIOPSIS SCHENKIANA

Stages in the infection of the algal cell were not found in our material. They have, however, been excellently described by Zopf (l.c.). Very early in the development of the thallus, the chloroplast of the alga contracts and undergoes a progressive dissolution. Destruction is initiated in the immediate vicinity of the young thallus and the chloroplast and nucleus are very soon fragmented. The parts of the chloroplast become noticeably narrower, lobed and constricted, as indicated in FIG. A. As destruction continues, the two halves of the chloroplast seem drawn towards the fungus thallus and destroyed. Ultimately, the entire structure is reduced to an irregular, hyaline, gelatinous-appearing mass lying close to and extending out from either side of the body of the parasite. Whatever substance is active in the disintegration of the chloroplast is apparently of great potency and readily diffusable from cell to cell, since chloroplasts in cells remote from the infected one, but in the same filament, are obviously affected. These in contrast to chloroplasts in nearby, uninfected filaments, are distorted and contracted. Furthermore, the bright green color is soon changed to a yellow-green. Contraction and discoloration of the chloroplasts of entire filaments were so constantly associated in our material with infection by the *Olpidiopsis* that when such algal plants were found, one could be absolutely certain that somewhere in the filament there was a thallus of the fungus. This extreme and powerful toxicity is in striking contrast to the action of many pathogens of higher plants, such as leaf parasites, where the interloper actually seems to exert a preserving influence on cells not immediately in the area of infection. There is no induced hyperplasia and usually little hypertrophy of the host cells. Occasionally, cells containing old parasites became somewhat enlarged in the region of the parasite, but this was not a typical reaction.

The young thallus is at first ellipsoidal; its cytoplasm is hyaline, glistening, and bears a few granules and vacuoles (FIG. A). As it becomes older, it increases in size, the protoplasm becomes granular, and there is formed a large central vacuole (FIG. B). Subsequently, a discharge tube develops which quickly grows
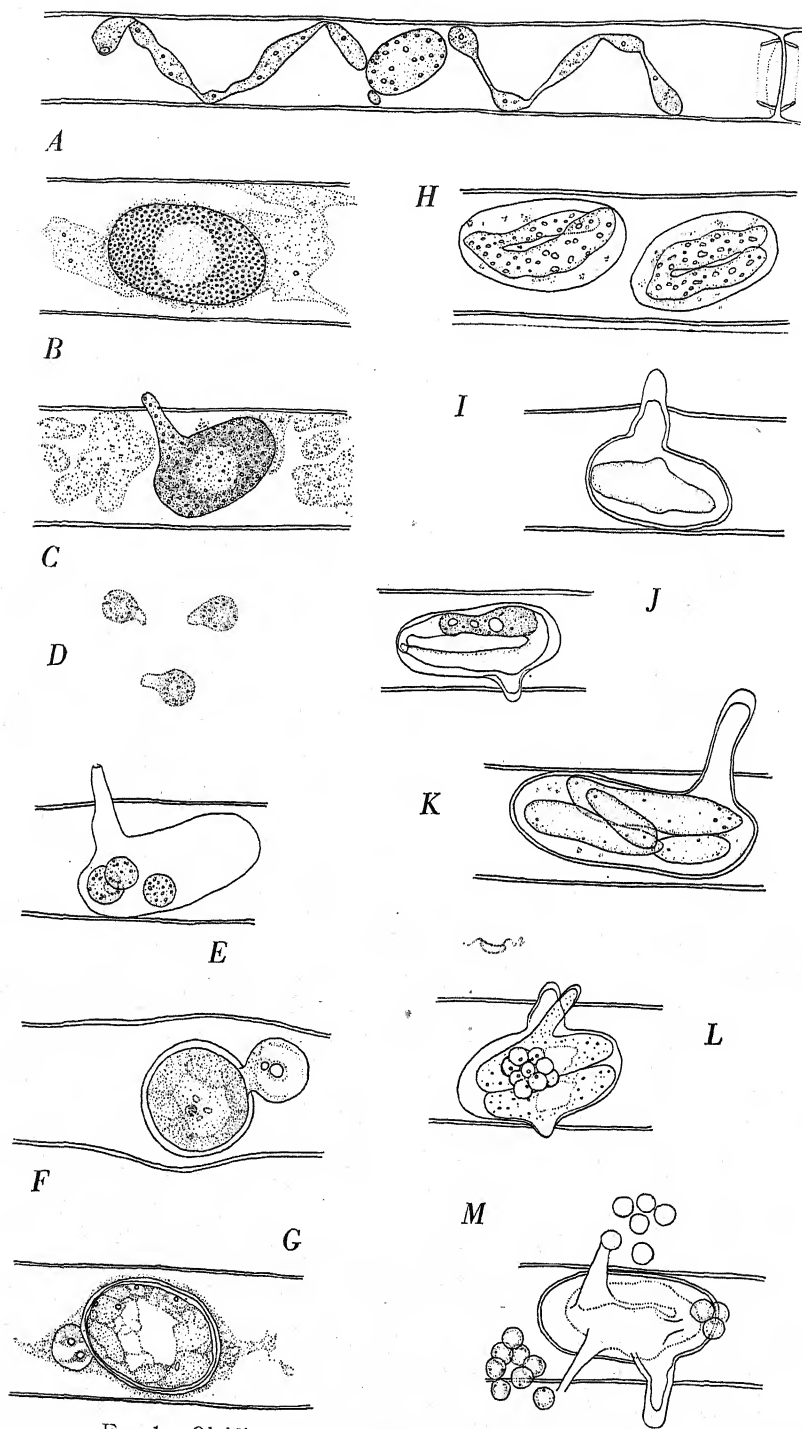
FIG. 1. *Olpidiopsis Schenkiana* and *Ectrogella Besseyi.*

through the wall of the *Spirogyra* cell (FIG. C). Cleavage of the contents of the sporangium into zoospores was not observed. It was noted, however, that the process took place before deliquescence of the tip of the single stout discharge tube. Zoöspore discharge in our material tended to support the claims of previous observers, including Zopf, that the zoöspores swim away without encystment. To be sure, there may be a period of amoeboid activity at the mouth of the tube, but there was no evidence that the spores ever encysted, as in *Achlya,* to emerge later as motile bodies, as Scherffel (8) has stated sometimes occurs. Observations on the hyperparasite, to be described later, may serve to explain this apparent discrepancy. The zoöspores are of the laterally biflagellate type, $8 \times 6 \mu$, with unequal, oppositely directed flagella. In addition to swimming, they may undergo periods of marked amoeboid movement (FIG. D). Occasionally they may become quiescent in the sporangium, after having failed to escape at the moment of discharge (FIG. E).

Resting spore formation was also observed. Formation of these bodies is preceded by a sexual process entirely similar to that found in other species of the genus and involves the conjugation of two thalloid gametangia. One, the receptive thallus, after receiving the contents of the other, steadily enlarges and becomes invested with a somewhat thickened, smooth wall (FIGS. F, G). An irregular, large central vacuole forms in the content which, in our material, occasionally tended to pull away from the resting spore wall (FIG. G). According to Zopf, this resting structure becomes converted into a zoösporangium at germination.

A diagnosis of the Michigan material is included here.

Olpidiopsis Schenkiana Zopf. Zoösporangium ellipsoidal, the long axis 32–41μ, the short axis 17μ; wall smooth, colorless, forming a single lateral stout discharge tube 15–17μ in length which opens upon the deliquescence of a prominent papilla; zoöspores $8 \times 7 \mu$, with two oppositely directed flagella, amoeboid after emergence but not encysting, soon swimming away. Resting spore spherical or more commonly somewhat ellipsoidal, 19–24μ in longest diameter, with a thickened, hyaline, smooth wall which turns brownish with age; companion cell spherical, 10–10.6μ in diameter, smooth-walled.

Parasitic in *Spirogyra* sp., vicinity of Ann Arbor, Michigan, November 27, 1945; Feb. 13, 1948.

THE HYPERPARASITE OF OLPIDIOPSIS

Among the sporangial material of *O. Schenkiana*, there were some which were obviously parasitized by another Phycomycete. This hyperparasite was present only in the vegetative and sporangial stages.

From one to four thalli were found in a single sporangium of the host. When developing singly, the body was asymmetrical and bilobed (FIGS. H, J) whereas when more than one was present, the lobing tended to be suppressed and the shape ellipsoidal (FIG. K). The contents of the immature thallus were hyaline and bore numerous small vacuoles. At maturity, it becomes converted into a single zoösporangium bearing one to several narrow discharge tubes which penetrate the wall of both the host sporangium and the *Spirogyra* cell and extend for a varying distance outside in the water. The zoöspores are delimited within the sporangium, escape individually and rapidly, and, as in *Achlya,* encyst at once at the orifice of the discharge tube (FIG. M). After a variable period of encystment, usually less than an hour, they emerge as zoöspores of the secondary, biflagellate type (FIG. L). No further stages in the life history were observed.

It is quite evident from the figures in Scherffel's (8) and Domján's (3) papers that this hyperparasite was present in their material of *Olpidiopsis Schenkiana*. Scherffel has noted what he terms "secondary sporangia" within the empty sporangia of *Olpidiopsis,* and similar structures are shown by Domján. These are interpreted here as belonging to the hyperparasite. Furthermore, Scherffel's observations that the zoöspore discharge may sometimes be of the *Achlya* type can also be explained on the basis that he was actually observing this process in the hyperparasite.

The proper generic disposition of this fungus on the basis of thallus structure and non-sexual reproduction alone is difficult. Because of its tubular, lobed, or branched thallus, it strongly resembles a species of *Petersenia* or *Pontisma*. However, in these genera, zoöspore discharge is like *Olpidiopsis,* no *Achlya*-like

encysted spore stage occurring. In the Ectrogellaceae of the Saprolegniales, on the other hand, fungi are found which resemble closely the present one. In the genus *Ectrogella,* the thallus is tubular, or, as in the present fungus, if several are in a cell, ellipsoidal; the sporangia are provided with one to several discharge tubes. Furthermore, in zoöspore discharge there is a strong tendency for the emerged zoöspores to encyst immediately or very soon after escape. Comparison might also be made with *Aphanomycopsis.* Here, however, zoöspores are formed in only a single row in the sporangium, not a feature of our fungus.

In *Pythiella vernalis* Couch, a close resemblance is found to the *Spirogyra* parasite in both the manner of zoöspore discharge and host plant. Here, the zoöspores encyst at the orifice of the sporangium and later escape from the cysts as biflagellate structures. The fungus parasitizes *Pythium,* a filamentous Phycomycete. Our fungus differs from *Pythiella,* however, in having tubular, lobed sporangia rather than spherical ones. If resting spores had been present, the generic affinities of the *Olpidiopsis* parasite could be decided with more certainty. Until these are found and their method of formation studied, the generic disposition of our fungus is in some doubt. It seems best at this time to include it in *Ectrogella.*

By reason of the striking difference in host plant of our fungus from the algal-inhabiting species of *Ectrogella,* it is considered a new species. In naming it, we have chosen to honor one who has always had a keen interest in aquatic Phycomycetes, Ernst A. Bessey.

Ectrogella Besseyi sp. nov. Sporangia irregulariter tubularia, saepe lobata, interdum ellipsoidalia, longitudine 27–42 μ, diametro 13–17 μ, singula vel pauca in cellulis *Olpidiopsidis Schenkianae,* tubulas zoosporis emittientibus singulas vel multas formantia longitudine variantes; zoosporis numerosis, intra sporangia obviis, demum liberis et indurescentibus in gregibus immobilibus ad tubi emittientis aperturam, ex cellula induriuscula emergentibus ut zoosporis biflagellatis, 5 μ longis, 2.5 μ crassis; sporis perdurantibus non visis.
    Parasitica in thallis et sporangiis *Olpidiopsidis Schenkianae* Zopf, prope Ann Arbor, Michigan, Novem. 1945 et Feb. 1948.

Sporangium irregularly tubular, often lobed, sometimes ellipsoidal, 27–42 μ long by 13–17 μ in diameter, one to several in a host cell, forming one to several discharge tubes of varying length;

zoöspores numerous, delimited within the sporangium, upon discharge from the sporangium encysting and forming motionless clusters at the orifice of the discharge tube, emerging from the cyst as a laterally biflagellate zoöspore, $5 \times 2.5 \mu$. Resting spores not observed.

Parasitic in thalli and sporangia of *Olpidiopsis Schenkiana* Zopf, vicinity of Ann Arbor, Michigan. November, 1945; February, 1948.

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EXPLANATION OF FIGURES

Fig. *A*. Young vegetative thallus of *O. Schenkiana* in *Spirogyra* cell. Chloroplast shows characteristic early stages of degeneration. *B*. Mature vegetative thallus of *O. Schenkiana* prior to formation of escape tube and zoöspores. *C*. Formation of zoöspore escape tube by *O. Schenkiana*. *D*. Amoeboid zoöspores of *O. Schenkiana* shortly after their emergence from the zoösporangium. *E*. Zoösporangium of *O. Schenkiana*. *F*. Young resting spore of *O. Schenkiana* with attached antheridium, showing penetration tube. *G*. Mature resting spore of *O. Schenkiana* with attached antheridium. *H*. Cells of *O. Schenkiana* containing young, single, bilobed thalli of *Ectrogella Besseyi*. *I*. Parasitized cell of *O. Schenkiana* showing incompletely formed discharge tube. Zoöspores of *E. Besseyi* have escaped through tube located on underside. *J*. Similar to figure 9. A portion of cytoplasm is left in one lobe of the *Ectrogella* thallus after discharge of zoöspores. *K*. Four thalli of *E. Besseyi* parasitizing one *O. Schenkiana* thallus. The unopened discharge tube is that of the *Olpidiopsis*. *L*. Formation of discharge tubes of *E. Besseyi*. Encysted zoöspores and secondary zoöspore above are from another thallus. *M*. Formation of multiple discharge tubes and discharge of zoöspores by *E. Besseyi*. Zoöspores encyst at the mouth as in *Achlya*. Incompletely formed discharge tube at lower right is that of the host, *O. Schenkiana*.

KEYS TO THE ORDERS, FAMILIES, AND GENERA OF THE GASTEROMYCETES [1]

S. M. ZELLER *

These keys are presented with no claim for originality. They have been prepared with full use of published keys, helpful literature, and personal advice from colleagues. Undoubtedly the most helpful have been the publications of our late colleague, Dr. Ed. Fischer, especially his most recent monograph (1933). Others whose assistance and advice have been sought and are thankfully acknowledged are Dr. W. C. Coker, Dr. H. M. Fitzpatrick, Dr. W. H. Long, Dr. G. W. Martin, and Dr. D. P. Rogers. Publications by the following authors have been used for ready reference: Bataille (1923), Coker & Couch (1928), Cunningham (1942), Fischer (1933), Hollós (1904), Kambly & Lee (1936), Lohman (1927), Lohwag (1926), Long (1907), (1917), (1940), (1941 [2]), (1942 [2]), (1943 [3]), (1944), (1945), and (1946 [3]), Longnecker (1927), Malençon (1931), Martin (1936), (1941), Morgan (1889–1893), Pilat (1934), Rea (1942), Stevenson and Cash (1936), and White (1901), (1902).

The following keys include all of the genera considered acceptable by the author. Genera which have been reduced to synonymy or may be considered doubtful are not mentioned here. Some of them have been dealt with elsewhere [2] or will be given consideration later when certain of the genera will be treated specifically. The new orders, Gautieriales and Tremellogastrales, have been previously discussed.[2]

It is realized there are several ways to build keys. One method is to follow strictly along lines of phylogeny, while another is merely an implement of plant identification. The keys presented below are primarily for plant identification without particular emphasis on relationships, although both methods are employed. For that reason, factors that may be used to separate two families or genera may not be the outstanding character or characters of the unit, but merely a sure way to distinguish them.

It will be helpful if colleagues will offer criticism on the keys as a whole or in part and on the general plan of organization of the Gasteromycetes as presented here.

KEY TO ORDERS OF THE GASTEROMYCETES

I. Gleba or spore mass surrounded by, or extending out to, a peridium at maturity (rarely without peridium), with one to many cavities, or lacunae filled with gel or basidia-bearing hyphae or nests.

  A. Original structure of gleba maintained until maturity, mostly bulb-like, hypogeous (sometimes epigeous), rarely stalked.

    1. Gleba more or less fleshy, not cartilaginous; basidia in a true hymenium (except in *Melanogastraceae*) ....1. *Hymenogastrales*

    2. Gleba and/or peridium cartilaginous or gelatinous or both.

      a. Spores ellipsoid, fusoid; with longitudinal ribs (costate).

                              2. *Gautieriales*

      b. Spores spherical, ellipsoid, ovoid, echinulate or sculptured.

                              3. *Tremellogastrales*

      c. Spores bacillar, ellipsoid, smooth; basidia phalloid.

                              4. *Hysterangiales*

  B. Gleba disorganized or fallen apart at maturity; peridium usually opening at maturity.

    1. Gleba a powdery or pulpy spore mass at maturity (except *Arachniaceae*).

      a. Gleba chambered by the outgrowth of tramal plates or pegs, walls of chambers covered with at least a rudimentary hymenium of basidia.

        1'. Gleba interspersed with or surrounded by a pseudoparenchymatous, spongy receptacle, which finally through its extension elevates the gleba, or holds it on the inner wall of a hollow chamber; spore mass pulpy, usually slimy and odoriferous, without capillitium ....5. *Phallales*

        2'. Gleba without receptacle; spore mass powdery at maturity (except *Arachniaceae*), with some sort of capillitium.

          a'. Peridium with one or more gelatinous layers.

                              3. *Tremellogastrales*

          b'. Peridium without gelatinous layer ....6. *Lycoperdales*

    b. Gleba with symmetrically distributed basidia, or with basidia-
       bearing nests or lacunae arising through the dissolution of
       fundamental tissue, without a well organized hymenium (except
       Batarrea) ....7. *Sclerodermatales*
  2. Glebal cavities isolated from one another as hard or brittle peridi-
    oles or "egg-like" bodies, through the dissolution of the tissue
    lying between; peridioles adhering to the inside of the peridium
    or forcibly ejected ....8. *Nidulariales*
II. Gleba or spore mass until maturity on the under or inner side of a cen-
  trally stalked cap or peridium; basidia at first in a true hymenium.
<div align="right">9. *Podaxales*</div>

ORDER I—HYMENOGASTRALES

Fructifications *mostly* hypogeous, bulblike, occasionally pear- or spindle-shaped, stalked, or epigeous; rarely with a stemlike columella; peridium remaining indehiscent to maturity, seldom disintegrating early; gleba of one or more cavities, lacunae filled with gel or with basidia-bearing hyphae or nests, holding original structure to maturity; peridium and gleba essentially fleshy, not cartilaginous; conidiophores when present borne in hymenium with basidia (*Holocotylon*) or in a separate fructification (*Leucophleps,* conidial stage of *Leucogaster*).

KEY TO FAMILIES IN THE HYMENOGASTRALES

I. Fructification minute, with a single glebal cavity at maturity.
  A. Spores smooth (coralloid development of fructification).
<div align="right">Family 1. *Protogasteraceae*</div>
  B. Spores verrucose (campanulate development of fructification).
<div align="right">Family 2. *Gasterellaceae*</div>
II. Fructification with many cavities, or basidial nests, or gel-filled cavities.
  A. Gleba with basidial nests or gel-filled cavities, or cavities lined with
    a false or rudimentary hymenium ......Family 3. *Melanogasteraceae*
  B. Gleba with open cavities lined with a true hymenium.
    1. Spores smooth ....Family 4. *Rhizopogonaceae*
    2. Spores verrucose ....Family 5. *Hymenogasteraceae*
    3. Spores echinulate ....Family 6. *Hydnangiaceae*

PROTOGASTERACEAE

Fructifications subspherical, hypogeous, very small, uniloculate, coralloid development; cavities lined with a basidial hymenium; spores smooth.

One genus, *Protogaster*.

GASTERELLACEAE

Fructifications very small, depressed globose, epigeous, campanulate development; gleba finally uniloculate, but at times with one circle of cavities formed by vertical, centripetal invaginations which reach the center forming a false columella; cavities lined with a basidial hymenium; spores verrucose, dark.

KEY TO THE GENERA OF THE GASTERELLACEAE

I. Gleba at first and at maturity consisting of a single, hymenium-lined cavity ....1. *Gasterella*
II. Gleba at first with a percurrent columella around which is one circle of cavities formed by vertical, centripetal invaginations which reach the center forming a false columella, and break away again at maturity; finally uniloculate ....2. *Gasterellopsis*

MELANOGASTERACEAE

Fructifications subglobose, usually hypogeous, sometimes stipitate at maturity; gleba of lacunae with basidia in nests or in a rudimentary hymenium from the walls of jelly-filled or pseudo-parenchyma-stuffed cavities; gleba not becoming powdery; capillitium none.

KEY TO THE GENERA OF THE MELANOGASTERACEAE

I. Gleba black or brown with basidia scattered through gel-filled lacunae.
   A. Gleba black marbled with whitish veins ....*Melanogaster*
   B. Gleba dark brown, marbled with light brown veins.
      1. Spores ellipsoid, smooth ....*Alpova*
      2. Spores spherical, echinulate; peridium and septa with lactiferous ducts ....*Maccagnia* (Not known in North America)
      3. Spores citriform, rough with loose epispore, as in *Hymenogaster*.
         Chondrogaster (Not known in North America)
II. Gleba light colored to white.
   A. Fructifications not stipitate.
      1. Gleba lacunate, not chambered, hard.
         Corditubera (Not known in North America)
      2. Gleba chambered, with an irregular hymenium lining chambers filled with hyphal tissue or gel.
        a. Spores borne on basidia.
           1'. Spores ellipsoid, smooth, without a gelatinous sheath.
              Cremeogaster
           2'. Spores mostly spherical, pitted, covered by a gelatinous sheath ....*Leucogaster*
        b. Spores borne on conidiophores ....*Leucophleps*

B. Fructification stipitate; volvate stem; spores oblong, hyaline.
Torrendia (Not known in North America)

RHIZOPOGONACEAE

Fructifications subglobose, hypogeous or epigeous; peridium simple, with or without rhizomorphic fibrils over the surface; gleba fleshy, not cartilaginous, with open irregularly arranged cavities, or cavities diverging from the base or from a branched or simple columella; original structure of gleba maintained to maturity; basidia in true hymenia; spores smooth, tinted.

KEY TO THE GENERA OF THE RHIZOPOGONACEAE

I. Fructifications mostly hypogeous, with rhizomorphic fibrils over the surface of the peridium, hymenium of basidia and paraphyses.
  A. Gleba without columella or conspicuous dendroid tramal plates.
                                            Rhizopogon
  B. Gleba with columella or conspicuous dendroid tramal plates.
                                       Truncocolumella
II. Fructifications epigeous, with no superficial fibrils, hymenium of basidia, conidiophores, and paraphyses ....*Holocotylon*
III. Fructifications with hollow stems, epigeous; spores ellipsoid, smooth.
               Le Ratia (Not known in North America)

HYMENOGASTERACEAE

Fructifications globose, hypogeous, with or without rhizomorphic fibrils over the surface of the peridium; peridium simple; gleba fleshy, not cartilaginous, dark, with or without columella; with true basidial hymenium; spores dark, verrucose.

KEY TO THE GENERA OF THE HYMENOGASTERACEAE

I. Fructifications without columella.
  A. Spores verrucose, mostly citriform ....*Hymenogaster*
  B. Spores angular, as in *Leptonia* * ....*Richoniella*
II. Fructifications with dendroid or percurrent columella, spores verrucose, mostly citriform ....*Gymnoglossum*

HYDNANGIACEAE

Fructifications hypogeous, subglobose or subpileate; with or without a columella or stipe, with campanulate development;

* Editor's note: This genus of the Agaricaceae is included in *Rhodophyllus* by most modern authors.

gleba fleshy, not cartilaginous, maintaining original structure to maturity; with true basidial hymenium; sometimes with lactiferous ducts; spores echinulate, slightly tinted, usually spherical.

KEY TO THE GENERA OF HYDNANGIACEAE

I. Gleba without columella, or tissues without lactiferous ducts; spores subglobose.
    A. Spores thick-walled; gleba gelatinous, cavities filled with spores.
                                        Sclerogaster
    B. Spores with thinner walls; gleba fleshy, not gelatinous..*Hydnangium*
II. Gleba with columella, and with lactiferous ducts in some or all tissues ....*Arcangeliella*
III. Gleba with columella or stipe; tissues without lactiferous ducts but always with islands of pseudoparenchyma ....*Elasmomyces*

ORDER II—GAUTIERIALES

Fructifications hypogeous, sessile; peridium wanting or present, when present stupose, loosely filamentous or pseudoparenchymatous; gleba gristly translucent and white when fresh, becoming brittle and brownish as spores mature; columella from a basal rhizomorph; basidia in a hymenium; septa usually gelatinous-cartilaginous, of gelified hyphae; basidiospores of various shapes, mostly broad fusiform, verrucose or longitudinally costate, brown.

One family, *7. Gautieriaceae,* with characters of the order. There is one genus, *Gautieria.*

ORDER III—TREMELLOGASTRALES

Fructifications hypogeous or epigeous, mostly sessile; peridium of two or more layers, the outer of fundamental tissue, the inner of a gelatinous nature, continuous or interrupted by sutures of fundamental tissue; gleba centripetally developed, pulverulent at maturity; columella simple or wanting; spores spherical, echinulate, verrucose or cristate.

KEY TO THE FAMILIES OF THE ORDER TREMELLOGASTRALES

I. Peridium with a gelatinous inner layer interrupted by sutures of fundamental tissue; spores spherical, echinulate or cristate.
                           Family 8. *Tremellogasteraceae*
II. Peridium with a simple, continuous gelatinous inner layer; spores spherical, minutely verrucose ....Family 9. *Gastrosporiaceae*

TREMELLOGASTERACEAE

Fructifications hypogeous or epigeous, mostly sessile; peridium of two or more layers, the outer of fundamental tissue, the inner of a gelatinous nature, interrupted by sutures of fundamental tissue; gleba centripetally developed, pulverulent at maturity; spores spherical, echinulate or cristate.

KEY TO THE GENERA OF THE TREMELLOGASTERACEAE

I. Inner layer of the peridium very thick, with branched sutures of fundamental tissue dividing the gelatinous portion of the inner peridium radially and periclinally into two more or less definite layers; spores spherical, echinulate ...*Tremellogaster* (Not known in North America)
II. Inner layer of the peridium thinner, of one gelatinous layer interrupted by radial sutures of fundamental tissue; spores spherical, echinulate and cristate or cristate only ...*Clathrogaster* (Not known in North America)

GASTROSPORIACEAE

Fructifications hypogeous, subglobose, from a single rhizomorph; peridium duplex, outer layer thin, of fundamental tissue (fibrous); inner layer gelatinous, continuous; columella simple; gleba pulverulent or deliquescent at maturity; spores spherical, minutely verrucose, slightly tinted or nearly hyaline.

One genus, *Gastrosporium,* not found in North America.

ORDER IV—HYSTERANGIALES

Fructifications mostly hypogeous, globose or elongate, mostly from rhizomorphic strands; peridium simple or with 2 to 3 layers, or with an inner gelatinous layer (tramal peridium); gleba cartilaginous, gelatinous; basidia phalloid; spores smooth, ellipsoid to bacillar; tramal structure radiating from the base or as continuations from the mycelial strands, diverging from sterile base, or from gelatinous or cartilaginous columella. Rarely with a percurrent columella (*Rhopalogaster*).

KEY TO FAMILIES IN THE HYSTERANGIALES

I. Tramal peridium not continuous.
   A. Tramal peridium not well developed or if so, cartilaginous, thin, interrupted by fertile or infertile cavities which are usually not filled by peridial tissue ....Family 10. *Hysterangiaceae*

B. Tramal peridium thick, gelatinous, interrupted by thin plates (sutures) of peridial tissue having unbroken connection with the fundamental peridium and sectors of the gleba..Family 11. *Protophallaceae*
II. Tramal peridium continuous, thick, gelatinous..Family 12. *Gelopellaceae*

HYSTERANGIACEAE

Fructifications hypogeous, mostly with rhizomorphic strands or heavy mycelial spawn; peridium simple or with 2 or 3 layers; gleba cartilaginous, gelatinous, tramal structure radiating from the base or from a gelatinous columella (columella percurrent in *Rhopalogaster*); spores smooth, ellipsoid, or bacillar.

KEY TO GENERA OF THE HYSTERANGIACEAE

I. Fructifications with prolonged, tapering, stalk-like sterile base, or extended into a branched, but not percurrent, columella.
  A. Thicker branches of the columella not dividing the gleba into sharply delimited sectors.
    1. Fructifications not stalked ....*Hysterangium*
    2. Fructifications with short stalk, extending into the gleba as a hemispherical, sterile base ....*Jaczewskia*
  B. Thicker branches of the columella dividing the gleba into sharply delimited sectors; fructifications with a prolonged, tapering, stemlike sterile base, pear-shaped ....*Phallogaster*
II. Fructifications with a stalk-like, unbranched percurrent columella.
                                                  Rhopalogaster

PROTOPHALLACEAE

Fructifications subglobose, hypogeous or epigeous; peridium usually thin, of primary tissue covering a thick gelatinous tramal peridium which is interrupted by radial sutures having unbroken connection with the peridium and gleba, which is gelatinous or cartilaginous, olivaceous or brownish, usually sectored by gelatinous plates radiating from the base or from a columella; cavities empty then filled with spores; basidial hymenium lining cavities; spores small, bacillar, olivaceous.

KEY TO GENERA OF THE PROTOPHALLACEAE

I. Gleba a powdery mass at maturity ....*Calvarula*
II. Gleba gelatinous-cartilaginous at maturity ....*Protubera*

GELOPELLACEAE

Fructifications subglobose, hypogeous; peridium thin, filamentous, surrounding a thick, continuous, gelatinous layer (tramal peridium); gleba dark, cartilaginous or gelatinous; cavities lined with basidial hymenium; columella simple or branched (pendant in *G. hahashimensis*); spores small, smooth, colored.

One genus, *Gelopellis,* not known in North America.

ORDER V—PHALLALES

Fructifications at first with a gelatinous universal veil, which is left at maturity as a cupulate volva at the base of a subspherical or ovoid, or a latticed or stem-like, pseudoparenchymatous receptacle which may be with or without a cap; gleba usually mucilaginous at maturity, surrounded by the receptacle, lying between its branches, or on the exterior of the cap or on the modified upper portion of the stem; spores bacillar, smooth.

KEY TO THE FAMILIES OF THE PHALLALES

I. Receptacle simple, hollow, ovoid ....Family 13. *Claustulaceae*
II. Receptacle stalk-like, hollow, cylindrical or fusiform, with or without a bell-shaped cap; gleba borne on exterior of cap or on the outside of a modified upper portion of the stem ....Family 14. *Phallaceae*
III. Receptacle latticed, lobed, or with irregular branches, stalked or stalkless; gleba surrounded by the receptacle or lying between its arms.
Family 15. *Clathraceae*

CLAUSTULACEAE

Fructifications spherical, smooth; peridium of 2 layers, the outer rind thin, of filamentous tissue, inner layer gelatinous, continuous; receptacle a hollow, indehiscent, pseudoparenchymatous sphere; gleba lining the interior of the receptacle, confined to one layer of cells; spores smooth, ellipsoid.

One genus, *Claustula,* not known in North America.

PHALLACEAE

Volva cupulate or sheathing, of 2 layers, the outer thin rind of filamentous primary tissue, the inner a thick, continuous, gelatinous layer; gleba surrounding the upper part of the receptacle;

receptacle porous, stalk-like, with or without a bell-shaped cap and sometimes with a continuous or meshy indusium; spores olivaceous or greenish, smooth, small, bacillar.

KEY TO THE GENERA OF THE PHALLACEAE

I. Receptacle simple, stalk-like, without a freely hanging campanulate cap, upper part wholly covered by a spore mass (gleba) or covered in a girdling zone only.
    A. Gleba-covered part of the receptacle without a pseudoparenchymatous sheath or reticulum.
        1. Receptacle in young stages not percurrent in the volva; fructifications growing on wood.
            Xylophallus (Not known in North America)
        2. Receptacle in young stages percurrent in the volva; fructifications growing on the ground; buttons hypogeous.
            a. Gleba covering a ring-like zone on the receptacle, below a sterile tip ....*Staheliomyces*
            b. Gleba covering most of the upper part (head) of the receptacle ....*Mutinus*
    B. Glebal part of the receptacle covered by the pseudoparenchymatous sheath or reticulum.
        1. Sheath closely fitting, smooth, granulose, papillose or netted.
            Jansia (Not known in North America)
        2. Glebal part of the receptacle covered by a loosely fitting pseudoparenchymatous reticulum.
            Floccomutinus (Not known in North America)
II. Receptacle composed of a hollow stem and a campanulate cap upon the outer surface of which the mass of spores (gleba) is borne.
    A. Indusium or reticulum not present at maturity.
        1. Cap and gleba continuous over the apex of the receptacle; cap gelatinous, fastened at lower edge to the stem.
            Aporophallus (Not known in North America)
        2. Apex of the receptacle covered by a pseudoparenchymatous cap or disk; gleba throughout pierced by numerous pseudoparenchymatous plates or strands, extending from the upper end of the stem or cap ....*Itajahya*
        3. Apex of the receptacle with an uncovered pore into the hollow of the receptacle, edge of the cap free; gleba not pierced by plates to the upper surface, but underlaid by a meshiness over the cap.
            Phallus
    B. Stem of the receptacle with a pseudoparenchymatous reticulum (indusium) hanging from under the cap.
        1. Reticulum (indusium) very short, collar-like, hidden under the cap; the latter perforated, lattice-like.
            Echinophallus (Not known in North America)
        2. Indusium at maturity longer than the cap, mostly net-like; cap not perforated ....*Dictyophora*

CLATHRACEAE

Volva at first enclosing the whole fructification, of 2 layers, the outer a thin filamentous layer, the inner a thick gelatinous layer (tramal peridium) interrupted by thin plates or sutures of primary, filamentous tissue making connection with the outer layer of the volva and sectors of the gleba; receptacle latticed, or with coral-like ramifications, stalked or sessile; gleba surrounded by portions of the receptacle or lying between its branches, dark olivaceous; spores small, bacillar, smooth.

KEY TO THE GENERA OF THE CLATHRACEAE

. Receptacle latticed or as meridian-like branches over the apex, branches fastened or with free ends at apex, like spokes of a wheel or like the upward rays on a crown; stalked or sessile.

A. Branches of receptacle solid, thick, composed of many layers of chambers.

1. Receptacle sessile, branches bound together lattice- or net-like.
Clathrus

2. Receptacle sessile, with meridian-like branches bound together at apex only.

   a. Walls of the innermost chamber of receptacle branches not opening at maturity ....*Colonnaria*

   b. Walls of innermost chamber of receptacle branches finally parting as wing-like appendages.
   Blumenavia (Not known in North America)

3. Receptacle stalked, with arms free at apex.
Aseroë (Not known in North America)

B. Branches of receptacle slender, terete, stalked, or bandlike, a single tube, or composed of very few layers of chambers.

1. Receptacle with branches bound together lattice-like.

   a. Receptacle long stalked; the latticed upper part hemispherical to spheroid, with small meshes ....*Simblum*

   b. Receptacle tapering downward, sessile or short stalked; lower meshes narrow, strongly elongated vertically, upper isodiametric, very small ....*Colus* (Not known in North America)

2. Receptacle with only vertical branches bound together at the apex.

   a. Receptacle sessile ....*Laternea*

   b. Receptacle stalked ....*Pseudocolus*

3. Upper end of receptacle with extended arms.

   a. Branches of the receptacle coming off vertically from the wall of the stem ....*Lysurus*

   b. Receptacle branches arising from the edge of a seam or an orbicular extension of the upper end of the stem and spreading horizontally at maturity.
   Aseroë (Not known in North America)

II. Receptacle stalked, beset by irregular coral-like processes extending radially from the upper end and between which the spore masses (gleba) lie ....*Kalchbrennera* (Not known in North America)

ORDER VI—LYCOPERDALES

Fructifications mostly epigeous, sessile, single or in groups on a stromatic layer, rarely substipitate, globose, pyriform, etc.; peridium 2–4-layered, dehiscing by an apical pore, by several pores, by irregular or stellate cleavages, or crumbling at maturity; gleba wholly fertile or sterile below, becoming a powdery mass or chambers breaking apart and forming at maturity small hollow peridioles; basidia borne in a hymenium; capillitium present (except in *Arachniaceae*).

KEY TO THE FAMILIES OF THE LYCOPERDALES

I. Whole peridium brittle, disintegrating at maturity; glebal chambers remaining intact but their walls scissile allowing the chambers to fall apart as peridioles, as fine sand-like particles ......Family 16. *Arachniaceae*
II. Endoperidium persistent; glebal chambers disintegrating into a powdery mass at maturity.
   A. Exoperidium not opening stellately at maturity (*Mycenastrum* possible exception).
      1. Peridium 2-layered, dehiscing by an apical stoma or breaking irregularly.
         a. Threads of capillitium smooth, branched, with or without a conspicuous, thick, main filament.
            1'. Fructifications not on a stroma ..Family 17. *Lycoperdaceae*
            2'. Fructifications singly or many on a stroma.
                    Family 18. *Broomeiaceae*
         b. Threads of capillitium with small, short, pointed (spine-like) side branches ....Family 19. *Mycenastraceae*
      2. Peridium 2–3-layered, indehiscent or rupturing irregularly at apex; capillitium unbranched ....Family 20. *Mesophelliaceae*
   B. Exoperidium opening stellately at maturity.
                    Family 21. *Geastraceae*

ARACHNIACEAE

Fructifications epigeous, small; peridium thin, fragile, breaking irregularly or crumbling at maturity to liberate the peridioles; gleba made up of numerous spherical chambers lined with a hymenium, forming at maturity a mass of minute, separate peridioles which are like grains of sand; capillitium and sterile base none; spores smooth.

KEY TO GENERA IN THE ARACHNIACEAE

I. Fructifications sessile, columella wanting ....*Arachnion*
II. Fructifications stipitate, columella present ....*Araneosa*

LYCOPERDACEAE

Fructifications single or in groups, mostly epigeous, subglobose to pyriform or nearly stipitate; gleba wholly fertile, or sterile below; outer peridium mostly a layer of pseudoparenchyma, rarely with a rind that is skin-like or permeated with soil particles, wholly or partially disintegrating at maturity, laying the inner peridium bare; inner peridium usually papery and thin, rarely corky and thick, usually dehiscing by an apical pore; rarely (in *Lycoperdopsis*) the two layers adhere to each other forming a simple pseudoparenchymatous rind, usually opening by an apical pore; capillitium well developed, sometimes falling into pieces.

KEY TO GENERA IN THE LYCOPERDACEAE

I. Peridium of a loosely interwoven endoperidium and closely adhering pseudoparenchymatous exoperidium.

  Lycoperdopsis (Not known in North America)
II. Peridium with a sharply differentiated, thicker textured endoperidium which is laid bare at maturity by sloughing the exoperidium.
  A. Endoperidium dehiscing by an apical pore (sometimes basal in *Disciseda*).
   1. Threads of capillitium smooth, more or less symmetrical, without a conspicuous main stem.
    a. Exoperidium wholly disintegrating.
     1'. Capillitium accompanied by membranes at maturity.
      Morganella
     2'. Capillitium not accompanied by membranes at maturity.
      Lycoperdon
    b. Exoperidium like a rind, or skin-like, or permeated with soil particles, and finally separating (circumscissilely) from a discoid or scutellate base exposing the endoperidium ....*Disciseda*
   2. Threads of capillitium very much branched, with a conspicuous main stem and slender branches with tapering pointed ends; spores with long pedicels ....*Bovistella*
  B. Endoperidium without apical pore, dehiscing by irregular rupture.
   1. Threads of capillitium smooth, without conspicuous main stem or conspicuous branching.
    a. Capillitium smooth or granular, broken, leaving blunt ends, sparingly branched.

1'. Endoperidium breaking up and falling away, usually leaving a sterile base covered by a definite membrane; capillitium threads usually broken ....*Calvatia*
2'. Endoperidium cartilaginous, very thin above, splitting into several irregular tooth-like segments at apex ..*Arachniopsis*
b. Capillitium threads simple, smooth, short with sharp ends.
Bovistoides (Not known in North America)
2. Threads of capillitium much branched with main stem and branches with long tapering pointed ends; free, not attached.
a. Capillitium threads not thickly interwoven, dispersing readily when exposed ....*Bovista*
b. Capillitium threads densely interwoven into balls .....*Lanopila*

BROOMEIACEAE

Fructifications singly or many on a stroma, mostly ovoid, hemispheric, or subglobose; exoperidium thin, wholly or partly disintegrated at maturity, endoperidium papery or thickish, laid bare at maturity, opening by an apical pore; capillitium present, threads more or less symmetrical, without a conspicuous, thick, main filament.

KEY TO THE GENERA OF THE BROOMEIACEAE

I. Fructifications more or less singly on a stroma, somewhat hemispheric ....*Lycogalopsis*
II. Fructifications many on a stroma, mostly ovoid.
A. Stroma thick, stalked, or columnar.
Broomeia (Not known in North America)
B. Stroma sessile, resupinate, or patellate ....:....*Diplocystis*

MYCENASTRACEAE

Fructifications large, subglobose to depressed globose; peridium duplex, exoperidium thick, spongy, smooth or areolate, endoperidium thick and leathery, or thin and membranaceous; capillitium branched with short pointed spines; spores spherical to ellipsoid, verrucose.

KEY TO THE GENERA OF THE MYCENASTRACEAE

I. Outer peridium smooth, thick, spongy, drying thin, fragile; inner peridium thick, corky, splitting stellately at maturity ....*Mycenastrum*
II. Outer peridium thick, coriaceous, areolate with irregular pyramidal sections; inner peridium thin ....*Calbovista*

MESOPHELLIACEAE

Fructifications hypogeous or epigeous, singly or several in a stroma; peridium usually 2–3-layered, indehiscent or rupturing irregularly at the apex; capillitium unbranched; spores globose or ellipsoid, variously roughened or with a gelatinous sheath.

KEY TO THE GENERA IN THE MESOPHELLIACEAE

I. Spores globose, echinulate, reticulate, or verrucose.
  A. Gleba with a sterile base ....*Radiigera*
  B. Gleba without a sterile base.
    a. Endoperidium without apical dehiscence ....*Abstoma*
    b. Endoperidium dehiscing by a simple stoma or lacerated opening ....*Bovistina*
II. Spores ellipsoid, smooth or irregularly roughened.
  A. Gleba with a large, hard, central core.
                Mesophellia (Not known in North America)
  B. Gleba without a central core.
                Castoreum (Not known in North America)

GEASTRACEAE

Fructifications at first hypogeous, or epigeous from the first, rounded or stalk-like below; peridium duplex; outer peridium 2–3-layered, pseudoparenchymatous layer within surrounded by a fibrous layer, at maturity opening out stellately ('in *Trichaster* the inner and outer peridium opening together); inner peridium papery thin, loosening from the outer peridium and dehiscing by a pore, or irregularly, or by many pores; gleba with a sterile columella from which the tubular chambers radiate.

KEY TO THE GENERA IN THE GEASTRACEAE

I. Endoperidium exposed as a whole at maturity, opening by a single, or rarely several pores.
  A. Endoperidium sessile or on a short stalk ....*Geastrum*
  B. Endoperidium on several thin stalks, with several pores above.
                    Myriostoma
II. Endoperidium disintegrating at maturity.
  A. Endoperidium with a prominent sterile base; columella soft, weak.
        Geasteroides Long (Not *Geastroides* Battarra)
  B. Endoperidium and exoperidium remaining joined and opening stellately together; without a sterile base; columella hard, sub-woody.
        Trichaster (Not known in North America)

ORDER VII—SCLERODERMATALES

Fructifications mostly epigeous; sporocarp sessile, on a false stem, or if stipitate, borne entirely above the stem or its expanded summit; peridium of 1–4 layers, dehiscing by an apical stoma, or by irregular fissuring or circumscissilely; gleba pulverulent at maturity, with or without capillitium; basidia symmetrically distributed or in nests or cavities arising through the dissolution of the tissue, without a well organized hymenium (except possibly in *Batarrea*).

KEY TO THE FAMILIES OF THE SCLERODERMATALES

I. Peridium mostly simple.
  A. Capillitium wanting or rudimentary.
    1. Gleba mostly veined or lacunate, developing internally or centrifugally ....Family 22. *Sclerodermataceae*
    2. Gleba with veins definitely extending centripetally from the peridium ....Family 23. *Sedeculaceae*
    3. Gleba breaking up into small peridioles..Family 24. *Pisolithaceae*
  B. Capillitium well developed; gleba entirely homogeneous.
                      Family 25. *Glischrodermataceae*
II. Peridium with distinct exo- and endoperidium.
  A. Sporocarp with a distinct, firm or gelatinous stalk; endoperidium persistent, papery, breaking away from the exoperidium at maturity.
    1. Stalk firm, fibrous or woody ....Family 26. *Tulostomataceae*
    2. Stalk made up of anastomosed strands forming a rough, lacunose stem, usually quite gelatinous when fresh.
                      Family 27. *Calostomataceae*
  B. Sporocarp sessile; exoperidium thick, splitting more or less readily from the endoperidium, so as to form astral rays.
                      Family 28. *Astraeaceae*

SCLERODERMATACEAE

Fructifications mostly epigeous, rarely hypogeous or emergent, subglobose, sessile or with an irregular root-like stem; peridium mostly simple, rarely 2-layered, firm, rarely thin, membranous, breaking open irregularly or in lobes or decaying; gleba with sharply defined basidia-bearing sectors, which are partitioned from one another by sterile veins, and in which the basidia are regularly scattered through the tissue (rarely, if ever, with hymenium-lined cavities or with fascicled, nested basidia); basidia broadly club-shaped; gleba crumbling to a powder of spores and disintegrating tissues at maturity; spores usually sculptured; capillitium wanting, or rudimentary.

KEY TO THE GENERA OF THE SCLERODERMATACEAE

A. Fructifications sessile or with elongate root-like base.
  1. Peridium smooth, finely warted or coarse surface.
    a. Spores without a distinct hilum or pedicel ....*Scleroderma*
    b. Spores with a hilum or pedicellate ....*Pompholyx*
  2. Peridium spiny or coarsely pyramidally warted.
                 Caloderma (Not known in North America)
B. Fructifications with slender stem.
                 Pirogaster (Not known in North America)

SEDECULACEAE

Fructifications leathery, without sterile base or radicle; peridium thick, leathery above, almost obsolete and dehiscing below; gleba becoming powdery at maturity, with broad veins extending inward from the peridium; spores brown, pedicellate or with sterigmatal scar.

One genus, *Sedecula.*

PISOLITHACEAE

Fructifications mostly stalked, rarely sessile (in *Pisolithus*); stems root-like or hard and wood-like; sporocarps subglobose, pear-shaped, or hemispherical; peridium thin or layers not separating readily, breaking away irregularly; gleba dark, made up of roundish or irregular basidia-bearing sectors or peridioles which loosen and break away at maturity; capillitium none; spores colored, sculptured.

KEY TO THE GENERA OF THE PISOLITHACEAE

I. Sporocarp sessile or with a rootlike stalk; peridium thin ....*Pisolithus*
II. Sporocarp with a hard woodlike stalk; peridium thick, hard.
                 Dictyocephalos

GLISCHRODERMATACEAE

Fructifications subglobose, on a superficial mycelium; peridium simple, thin but hard and tough, opening by an apical pore; gleba with evenly distributed basidia (without sterile veins), capillitium arising from the inner side of the peridium (as in *Calvatia*); spores sculptured.

One genus, *Glischroderma,* found in Europe only.

TULOSTOMATACEAE

Fructifications at first hypogeous, sporocarp elevated by the prolongation of a basal tissue into a stout, fibrous, stemlike or cushionlike process; peridium duplex, outer layer partly evanescent, partly remaining as a cuplike volva at the base of the stem, inner layer thin, dehiscing by an apical pore, several pores or circumscissilely; gleba without chambers or chambered by the labyrinthine separation of the tissues from one another; basidia regularly and evenly distributed in the glebal tissue or forming a rudimentary hymenium on the walls of chambers; capillitium well-developed, attached to the inside of the peridium; spores variously sculptured.

KEY TO THE GENERA OF THE TULOSTOMATACEAE

I. Basidia borne irregularly in fascicles or nests; endoperidium dehiscing irregularly or through a stoma.
  A. Stem more or less readily breaking from the sporocarp, as out of a socket.
    1. Sporocarp dehiscing by an apical stoma ....*Tulostoma*
    2. Sporocarp dehiscing by a roughly stellate stoma ....*Schizostoma*
    3. Sporocarp dehiscing irregularly ....*Queletia*
  B. Exoperidium continuous with the outer layer of the stem, endoperidium forming more or less of a cup or collar on the margin of the expanded apex of the stem; stem not volvate ....*Phellorinia*
  C. Exoperidium not continuous with the stem, sporocarp seated on the expanded apex of the stem; stem volvate ....*Chlamydopus*
II. Basidia borne in an elementary hymenium lining cavities; endoperidium dehiscence circumscissile or through numerous pores ....*Battarrea*

ASTRAEACEAE

Fructifications epigeous or at first hypogeous, sessile; peridium of several layers, the outer two or three becoming the very heavy exoperidium, which dehisces stellately; the endoperidium thin, membranous; columella none; gleba separated by delicate sterile veins into basidia-bearing sectors in which the basidia are regularly distributed throughout the context; basidia broadly clubshaped; spores spherical, sculptured; capillitium none.

One genus. *Astraeus.*

CALOSTOMATACEAE

Fructifications epigeous or at first hypogeous, stalked with a root-like, lacunose basal process; peridium duplex, exoperidium

cartilaginous, extended below into a rootlike stalk and often cupulate around the base of the sporocarp; endoperidium cartilaginous, with an ornate apical stellate stoma below which the spore sac is suspended; gleba pulverulent; spores spherical or ellipsoid, smooth or sculptured.

One genus, *Calostoma*.

ORDER VIII—NIDULARIALES

Fructifications small, sessile, cupulate, campanulate or depressed globose; peridium of one to four layers, dehiscing by rupture of an epiphragm or lid over the top, or when this is absent, by irregular fissuring of the wall; gleba enclosed in one or many globose or lens-shaped peridioles; peridioles attached to the inner wall of the peridium by a mucilaginous secretion or by threadlike funiculi, escaping singly or they may be forcibly ejected from the exoperidium; capillitium none.

KEY TO THE FAMILIES OF THE NIDULARIALES

I. Exoperidium more or less urceolate and firm at maturity; glebal chambers (peridioles) egglike, remaining attached or free within outer peridium, empty ....Family 29. *Nidulariaceae*
II. Exoperidium collapsed at maturity; single spherical, glebal chamber violently discharged at maturity, filled with gel or gelatinous tissue.
Family 30. *Sphaerobolaceae*

NIDULARIACEAE

Fructifications epigeous, with hard peridium which opens cuplike at maturity; gleba with a few mostly flattish, rounded, closed chambers (peridioles), the hard walls of which are lined with basidial hymenium and in the mature fructification are isolated or freed from the cup-like fructification by ejection or by the deliquescence of the intervening tissue.

KEY TO THE GENERA OF THE NIDULARIACEAE

I. Peridioles without funiculus.
  A. Fructification roundish, without typical epiphragm ....*Nidularia*
  B. Fructification beaker-shaped, with epiphragm ....*Nidula*

II. Peridioles with funiculus; fructifications top-shaped, with epiphragm.
    A. Peridium composed of three layers, middle layer of pseudoparenchyma; spores mixed with filaments ....*Cyathus*
    B. Peridium composed of one layer, not pseudoparenchyma; spores not mixed with filaments ....*Crucibulum*

SPHAEROBOLACEAE

Fructifications tiny, spherical at first; peridium of several layers, of which the second inner layer is formed of turgescent cells appearing as a radial palisade; gleba of basidia-bearing sectors separated by sterile veins, or of basidia-bearing cavities formed by the splitting of tissues; the gleba becomes gelatinized at maturity and is ejected as a whole from the peridium.

KEY TO THE GENERA OF THE SPHAEROBOLACEAE

I. Basidia borne irregularly throughout the basidia-bearing sectors.
                                           Sphaerobolus
II. Basidia borne in hymenia on the walls of cavities ....*Nidulariopsis*

ORDER IX—PODAXALES

Fructifications epigeous, stalked or with percurrent columella, pileate at maturity, angiocarpic; stipe long or short, continued to the apex of the fructification as a columella; peridium simple or 2–3-layered at maturity, left at maturity in part as pileus, or volva, or annulus on the stem; gleba at first with hymenium of basidia covering the walls of chambers or pores or lamellae, persistent or pulverulent; capillitium wanting (except in *Podaxis*); spores colored.

KEY TO FAMILIES OF THE PODAXALES

I. Gleba not powdery at maturity ....Family 31. *Secotiaceae*
II. Gleba powdery at maturity ....Family 32. *Podaxaceae*

SECOTIACEAE

Fructifications mostly epigeous, stalked or sessile, at first campanulate, like an agaric button, angiocarpic; stalk continued above as a percurrent columella; peridium mostly as a cap covering the gleba, free at maturity or opening by a transverse slit; gleba chambered or with irregular pores or with anastomosing, lamel-

loid, tramal structures, dark brown to blackish, sometimes with cystidia, not becoming a powdery spore mass at maturity; spores dark, smooth or sculptured; capillitium none.

KEY TO THE GENERA OF THE SECOTIACEAE

I. Stems not volvate, not annulate; gleba brown.
  A. Fructifications short-stemmed or sessile; pileus mostly globose to depressed globose, spores smooth or sculptured ....*Secotium*
  B. Fructifications with long stems; pileus cylindrical to conic, slender, acute at apex; spores smooth ....*Galeropsis*
II. Stems volvate and/or annulate; spores black or very dark brown.
  A. Gleba lamelloid, radiating and hanging free from the margin of the expanded apex of the stem; stem volvate; spores black ..*Montagnea*
  B. Gleba of anastomosed lamellae, attached to the underside of the expanded top of the stem (pileus), free from the stem; stem annulate; spores black ....*Longula*
  C. Gleba of anastomosed to poroid lamellae, suspended from the lower surface of the pileus, free; stem with a cupulate or sheathing volva; annulus usually persistent; spores black (rusty black *in ours*).
Gyrophragmium

PODAXACEAE

Fructifications angiocarpic, like agaric buttons, epigeous at maturity, clavate, ovoid or fusiform, stalked or almost sessile; stem firmly fibrous, extending percurrently as a columella; peridium pileate, simple or plicately scaly, brittle, easily splitting, margin loosening from the stipe at maturity or opening by longitudinal splitting; gleba at first chambered by anastomosing tramal tissues or lamelloid tramal plates, powdery at maturity; basidia persisting in *Podaxis;* capillitium well developed as elaters, or wanting; spores dark, smooth.

KEY TO THE GENERA OF THE PODAXACEAE

I. Fructification sessile or nearly so; gleba without capillitium.
Endoptychum
II. Fructification with long stipe; gleba with capillitium (elaters) ..*Podaxis*

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THE SPECIES OF TYMPANIS OCCURRING ON PINUS [1]

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(with 7 figures)

The problem of species identification in the genus *Tympanis* has interested the senior author for a number of years and collections and cultures have been accumulated for the purpose of making a comparative study. Special interest is attached to the species occurring on pine because of the *Tympanis* canker described by Hansbrough (**1936**) and caused by a species that could not be identified at that time. Dr. Hansbrough generously placed a large number of his collections at our disposal, and after a critical study of the available material by the junior author, we have distinguished three species occurring on pine.

These species are recognized principally by the character of the primary ascospores, which are fusiform, one- or two-celled in *T. hypopodia,* subglobose in *T. pithya,* and elongate-fusiform to clavate-fusiform and one- to several-celled in *T. confusa.*

Thirteen species of *Tympanis* have, at various times, been reported as occurring on *Pinus.* Of these, five can be eliminated at once as not being true *Tympanis* species. These are *T. pithya* (Fr.) Sacc. which will be discussed in detail below, *T. amphibola* (Massal.) Karst. (= *Pragmopara amphibola* Massal.), *T. bacillifera* Karst. (= *Pragmopara bacillifera* (Karst.) Rehm), *T. Tautziana* Ruhland which appears to be a *Biatorella* from the description and certainly can be excluded from *Tympanis* on the basis of the red color, and *T. sepiaria* Karst. which cannot be placed satisfactorily at present. No material of this species has been seen, but since no secondary ascospores have been described and the de-

scription does not suggest a *Tympanis,* it can be excluded from this genus.

Two of the remaining names, *T. laricina* (Fckl.) Sacc. and *T. Buchsii* (Henn.) Rehm, may be disregarded as being the result of misdeterminations. *T. laricina* was originally described on *Larix* by Fuckel (**1870**) and the specimen in Fuckel Fung. Rhen. *2473* was cited. This specimen has been examined and appears to be distinct from any of the specimens on pine but does agree with other collections on *Larix*. *T. laricina* appears to be a distinct species occurring on *Larix* and any record on other hosts is probably due to a misdetermination. *T. Buchsii* was originally described by Hennings (**1903**) as occurring on *Abies*. No material of this species has been examined but studies of numerous collections and cultures of *Tympanis* species occurring on *Abies* indicate that they are distinct from the pine-inhabiting species. Therefore, it seems highly probable that reports of *T. Buchsii* on pine are also the result of misdeterminations.

Probably the best known and most frequently used name is *T. Pinastri* Tul. This name was given by Tulasne (**1865**) to a fungus that was unquestionably a *Tympanis* and was said to be on *Abies*. The name has since been applied more or less indiscriminately to any *Tympanis* found on conifers by many authors. However, it is clear from the Tulasnes' account that they considered their fungus to be *Cenangium Pinastri* Fries and their specific epithet is, therefore, based on the earlier Friesian name. Rehm (**1889**) has shown that *Cenangium Pinastri* Fries is not a *Tympanis* and has transferred it to *Tryblidiopsis*. He retained the Tulasne name for a species of *Tympanis* but this is not permissible under the International Rules. According to Article 54, *Tympanis Pinastri* Tul. must be considered as being based on Fries' type and therefore a synonym of *Tryblidiopsis Pinastri* (Fr.) Rehm. Thus, Tulasne's fungus is left without a name and the combination *Tympanis Pinastri* is invalid for any *Tympanis* species.

The descriptions of *T. farinacea* (Pers.) Rehm are so incomplete that it is impossible to decide whether or not the fungus belongs in *Tympanis*.

Of the remaining four names, the earliest is *T. pithya* (Karst.) Karst. which dates from 1867 when a specimen was issued in

Karsten's Fungi Fenniae *661* as *Patellaria pithya* Karst. with a printed description on the label.   According to Articles 36 and 37 of the International Rules this is a valid publication.

In the literature, *T. pithya* Karst. has been confused with *Cenangium pithyum* Fries.   Fries (**1822**) described *Cenangium pithyum* on *Pinus sylvestris* and cited the specimen in Fries Sclerom. Suec. *172*.   Through the kindness of the late Dr. D. H. Linder it was possible to examine a slide of this specimen which is labelled *Excipula pithya*.   This slide disclosed a fungus with filiform, many-septate ascospores.   There was no evidence of secondary ascospores and the asci were not *Tympanis*-like.   Evidently, therefore, *Cenangium pithyum* Fries is not a *Tympanis*.

Patellaria pithya Karst. was not based on *C. pithyum* Fr. but was published quite separately and based on the specimen in Karst. Fung. Fenn. *661* as noted above.   Examination of a slide from this specimen in the Farlow Herbarium, and later examination of the specimen in Kew Herbarium, has shown that this fungus is actually a *Tympanis*.   The size of the asci agrees well with the size stated in the description on the label and it has ellipsoid to subglobose primary ascospores.

Karsten (**1871a**) transferred the fungus to *Tympanis*.   He did not describe primary ascospores but he cited the specimen in Fung. Fenn. *661* and the size of the asci as stated in his description agrees with that found in the actual specimen.   The description given by Karsten (**1871b**) is evidently also based on the specimen in Fung. Fenn. *661*.

Confusion arose from Karsten's later description (**1871c**) in which, under the name *T. pithya,* he actually described *T. hypopodia* Nyl. and cited the specimen in Karst. Fung. Fenn. *754*. Karsten recognized that this was *T. hypopodia* for he cited it as a synonym, but he failed to realize that *T. pithya* and *T. hypopodia* were distinct species.

Saccardo (**1889**) unfortunately followed Karsten's later and erroneous account.   On the basis of the two-celled primary ascospores, Saccardo made the combination *Cenangella pithya* (Karst.) Sacc. and cited *Tympanis hypopodia* Nyl. as a synonym.   In the meantime, Fuckel (**1870**) had described a fungus which was really a *Tympanis* under the name of *Cenangium pithyum* Fr.

Saccardo, thereupon, created the combination *Tympanis pithya* (Fr.) Sacc. Although Saccardo was careful to point out that this was not *T. pithya* Karst. it was, of course, a later homonym of Karsten's name and invalid under the present-day rules even if Fries' fungus were a *Tympanis*.

The climax to the confusion came when Rehm (**1889**) wrote the name as *Tympanis pithya* (Fr.) Karsten, a combination which never existed. Karsten at no time made any reference to *Cenangium pithyum* Fries.

Thus, *Tympanis pithya* (Karst.) Karst. is a valid name for a species of *Tympanis* occurring on pine and of which the type is Karst. Fung. Fenn. *661; T. pithya* (Fr.) Sacc. is a synonym of *Cenangium pithyum* Fr. whatever it may prove to be; and *T. pithya* (Fr.) Karst. is an imaginary combination that never existed in fact.

Nylander (**1868**) described three species of *Tympanis* on pine which must be considered: *T. hypopodia, T. confusa,* and *T. amphiboloides* var. *hypopodiza*. This is the first really critical study of *Tympanis* and Nylander was the first and for many years the only one who made an attempt to study and describe the primary ascospores.

T. hypopodia was said to have two-celled primary ascospores 8–9 × 2–3 μ in asci 8–9 μ in diameter, becoming 10–11 μ in diameter when multi-spored. The type was collected by Karsten in 1859. A specimen in Karst. Fung. Fenn. *754* collected in 1860 has been examined. It is apparently not the type but it agrees in every respect with Nylander's description and is the basis of our concept of this species.

T. confusa was described by Nylander (**1868**) based on the specimen in Fries Sclerom. Suec. *336* under the name *Patellaria atrata* Fr. Nylander found that this was really a *Tympanis* and described the primary ascospores as elongate-fusiform, 1–3-septate, 16–25 × 3–4 μ in asci 16–18 μ in diameter. The multi-spored asci were said to be 125–150 × 24–25 μ. Through the kindness of Miss E. K. Cash it was possible to examine a slide from this specimen. We found primary ascospores exactly as described but the asci were narrower, the multi-spored asci being mostly 16–18 μ in diameter and occasionally up to 21 μ.
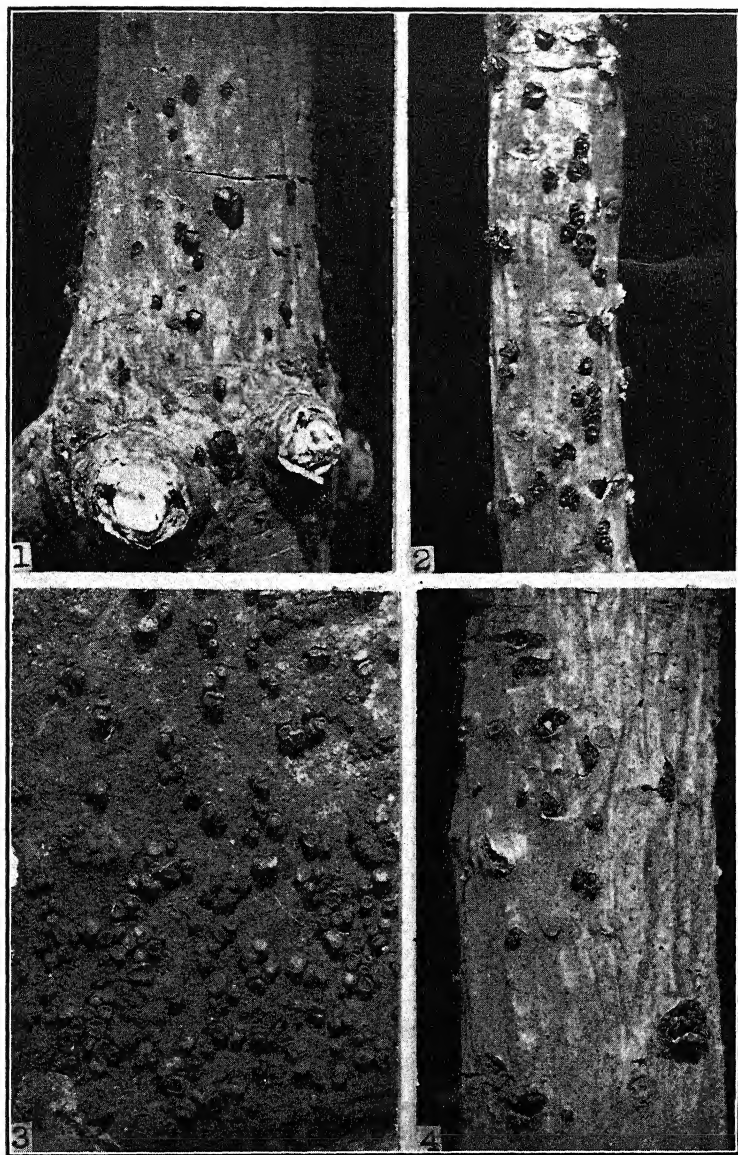
FIGS. 1–4. Species of *Tympanis*.

T. amphiboloides var. *hypopodiza* was also described by Nylander (1868) and there were only two points of difference between this and *T. confusa*. The primary ascospores were said to be about the same size but seven septate with an occasional longitudinal septum, and the asci were 11–15 μ in diam., with the multi-spored asci about 100–110 × 16 μ. Saccardo (1889) raised it to specific rank and transferred it to *Scleroderris* on the basis of the primary ascospores but it is unquestionably a true *Tympanis*. A specimen in Karsten Fung. Fenn. *753* labelled *T. amphiboloides* var. *hypopodiza* was examined and the asci were mostly 110–140 × 16–20–(22) μ with elongate-fusiform primary ascospores, mostly one to three septa but occasionally more. This specimen appears to be identical with *T. confusa* from Fries Sclerom. Suec. *336*.

In the species causing the canker of red pine described by Hansbrough (1936), the primary ascospores were said to be 11–25 × 3–5 μ and three- to nine-septate. This has been confirmed from an examination of Hansbrough's material and thus, as far as the primary ascospores are concerned, they match both the type of *T. confusa* and the specimen in Karst. Fung. Fenn. *753* labelled *T. amphiboloides* var. *hypopodiza*. However, the asci in Hansbrough's species were said to be 12–17 μ in diameter and this was also confirmed from examination of his specimens. It was unusual for an ascus to exceed 16 μ in diameter. This agrees with the size given in Nylander's description of *T. amphiboloides* var. *hypopodiza* but is narrower than the asci observed in the Fries and Karsten specimens.

Evidently, Nylander considered that there were two species and this possibility cannot be entirely ruled out. If so, the name *T. confusa* should be used for the species with mature asci 16–20 μ in diameter, whereas var. *hypopodiza* would require a new combination in *Tympanis* and be used for the species with mature asci mostly 13–16 μ in diameter. From the material examined, septation of the primary ascospores would be a wholly unreliable character for species differentiation and the distinction would rest solely on the difference in ascus size. It seems scarcely justifiable to recognize a species on an extreme difference of 3–4 μ in the width of the asci in only two specimens of an admittedly variable group. We have, therefore, decided to regard *T. confusa* and *T.*

amphiboloides var. *hypodiza* as synonyms, of which *T. confusa* is the valid name. Hansbrough's fungus is referred to this species and the description below is based principally on his material.

The difficulty of identifying these species is enhanced by the great similarity in many of the characters. The apothecia in all three species are black, sessile to substipitate, separate or cespitose, and about the same diameter. *T. confusa* does tend to be more regular in outline, more strongly erumpent, and substipitate, whereas *T. pithya* is usually sessile and strongly undulate. The apothecia of *T. hypopodia* are usually slightly smaller than those of the other two species. However, these characters all vary considerably in different collections, often differing according to their occurrence on young twigs or more mature bark. It is not possible to identify them by the gross appearance.

The consistency and tissue structure are identical in all three and are of no value in species differentiation.

The asci, as usually observed, are multi-spored, containing hundreds of minute, hyaline spores. These are actually secondary spores and might be regarded as conidia. They, also, are similar in all three species. It generally requires careful search to find the true or primary ascospores. They are indistinct and transitory, and frequently it is impossible to find them at all. We have found no evidence that they are ever discharged from the asci.

Hansbrough (**1936**) suggested that the asci should be considered physiologically mature when the primary ascospores have been formed but not morphologically mature until the secondary ascospores are formed. Since the asci do not appear to discharge at the primary ascospore stage it is questionable whether they are really physiologically mature at this time. It is certain that they are not morphologically mature at this stage and this is an important point in the identification of species.

When the asci are in the primary ascospore stage and for some time after the secondary ascospores have begun to form, the wall of the ascus is greatly thickened and gelatinized. As the ascus approaches maturity the wall becomes thinner until it is not noticeably thicker than in asci in related genera. If measurements are made on the thick-walled asci they will be found to vary considerably,

whereas in the fully mature thin-walled asci they are relatively constant.

The size of the mature asci is, thus, of considerable value as a diagnostic character. *T. hypopodia* can be recognized with reasonable certainty from the ascus size alone, which rarely exceeds 90μ in length and 12μ in width. In general, the asci of *T. pithya* are slightly longer and wider than those of *T. confusa,* but there is considerable overlap in size. When most of the asci exceed 15μ in diameter it is usually *T. pithya,* and when most of them are less than 15μ it is usually *T. confusa.* However, the two European collections cited above proved to be exceptions and often it is impossible to place a specimen with certainty from ascus size alone in these two species. Chief reliance must be placed on the primary ascospores as a means of separating these species.

Except in *T. confusa,* where there are rarely more than four primary ascospores, the asci usually contain eight. Nylander (1868) spoke of the asci being up to twenty-four-spored but we have not been able to confirm this. It does seem that in the early stages of the formation of secondary ascospores, the first bodies formed are larger than the ultimate secondary ascospores. Very rarely, broken asci have been observed from which these bodies were emerging. They were larger and more irregular in size and shape than the secondary ascospores, but the true primary ascospores were not apparent. It is believed that Nylander observed something like this. Asci at this stage are very confusing for they may seem to contain numerous globose spores or several, many-septate, elongated spores, but careful search will usually reveal the true primary ascospores in other asci.

Brefeld (1891) stated that the secondary ascospores arose by budding from the primary ascospores, and figured primary ascospores with secondary ascospores forming as buds on the ends. We have also observed this especially in *T. pithya,* where the primary ascospores sometimes appear to bear appendages, which have been interpreted as budding secondary ascospores.

In contrast to *Dermea* (Groves, 1946), where the conidial states proved to be of great diagnostic value in identification of species, in *Tympanis* the conidial fruiting bodies and conidia are very similar in all of the species. The fruiting bodies are usually

ovoid to cylindric, associated with more or less stromatic tissue, black, similar in consistency and tissue structure to the apothecia, and containing cavities lined with filiform conidiophores from which the conidia arise at the tip and along the sides. The conidia are indistinguishable in the various species and almost indistinguishable from the secondary ascospores, although they may be slightly larger and more variable than the latter.

Von Höhnel (1914) erected the genus *Pleurophomella* for the conidial states of *Tympanis* species, of which the type was *Pleurophomella eumorpha* (Penz. & Sacc.) v. Höhn., said to be the conidial state of *Tympanis Pinastri*. It is not possible to distinguish any of the conidial states of *Tympanis* species unless they are directly associated with the apothecia. If cultures can be obtained from the conidial state it may be possible to identify the species but such identifications are at best uncertain. *P. eumorpha* was originally described from *Pinus* and probably is the conidial state of one of the three species recognized in this paper but it is not possible to say to which one it belongs.

All three of these species have been studied in culture from isolations originating from both ascospores and conidia. When the asci are allowed to discharge on to agar, the whole mass of secondary ascospores forms a yeastlike colony which at first continues to grow by budding, but soon puts out a delicate fringe of hyphae. Conidia arise, not only by budding from other conidia, but also along the sides of the hyphae, forming a *Pullularia*-like colony. In older colonies, there is more extensive development of mycelium and sometimes a little whitish aerial mycelium is produced.

Cultures have been grown on two per cent malt extract agar and on sterilized twigs of the host. Conidial fruiting bodies are produced readily on agar by all three species. In *T. pithya* there is, perhaps, more tendency to produce irregular black stromatic masses which sporulate more rarely. In *T. hypopodia* the conidial fruiting bodies tend to appear later than in the other species, and to be slightly smaller and more scattered, but these characters are not clear cut and vary so much in different isolates of the same species that it does not seem possible to identify them by cultural characters alone.

On sterilized twigs they produce very little aerial mycelium but pycnidia are usually abundant and larger and more cespitose than observed in nature. Apothecia have been produced on the twigs from ascospore cultures of all three species and also from conidial cultures of *T. pithya*.

TECHNICAL DESCRIPTIONS

TYMPANIS PITHYA (Karst.) Karst. Hedw. **10**: 58. 1871. (not *T. pithya* Sacc. Syll. **8**: 583. 1889.)

> *Patellaria pithya* Karst. Fung. Fenn. No. *661*. 1867.
> *Cenangella pithya* Sacc. Syll. Fung. **8**: 588. 1889.

Apothecia erumpent, gregarious, separate or cespitose, usually in clusters of less than six, circular to strongly undulate, sessile, narrowed below, black, glabrous or occasionally slightly grayish-pruinose, 0.5–1.0 mm. in diameter, 0.3–0.7 mm. in height, hard, horny in consistency becoming more cartilaginous-fleshy when moist; hymenium concave to plane, dull black or shiny black, more fleshy than the excipulum, at first with a thick, somewhat infolded margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 1–2 μ in diameter, with the walls greatly thickened and gelatinized, becoming darker and thicker at the outside forming a rind-like excipulum, subhymenium not clearly differentiated; asci cylindric to cylindric-clavate, short-stalked, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multi-spored, (80)–95–140–(165) $\times$ (11)–14–18–(22) μ; primary ascospores hyaline, broadly ellipsoid to subglobose, one- or two-celled, uniseriate, 5–8 $\times$ 3–5 μ; secondary ascospores hyaline, cylindric to allantoid, one-celled, 2.0–3.0 $\times$ 1.0–1.5 μ; paraphyses hyaline, filiform, septate, simple or branched, 1.5–2.0 μ in diameter, the tips slightly swollen to 3.0 μ and embedded in a gelatinous matrix forming a brownish epithecium.

Conidial fruiting bodies erumpent, gregarious to scattered, separate or cespitose, black, glabrous, globose or more or less ovoid, sometimes laterally confluent, 0.2–0.3 mm. in diameter, 0.2–0.4 mm. in height, similar in consistency to the apothecia; tissue plectenchymatous, similar to that of the apothecia, in the upper part containing an ovoid to slightly elongated cavity, lined with conidiophores and tearing open at the top; conidiophores hyaline, filiform, septate, simple, about 25–80 $\times$ 2.0–2.5 μ; conidia hyaline, one-
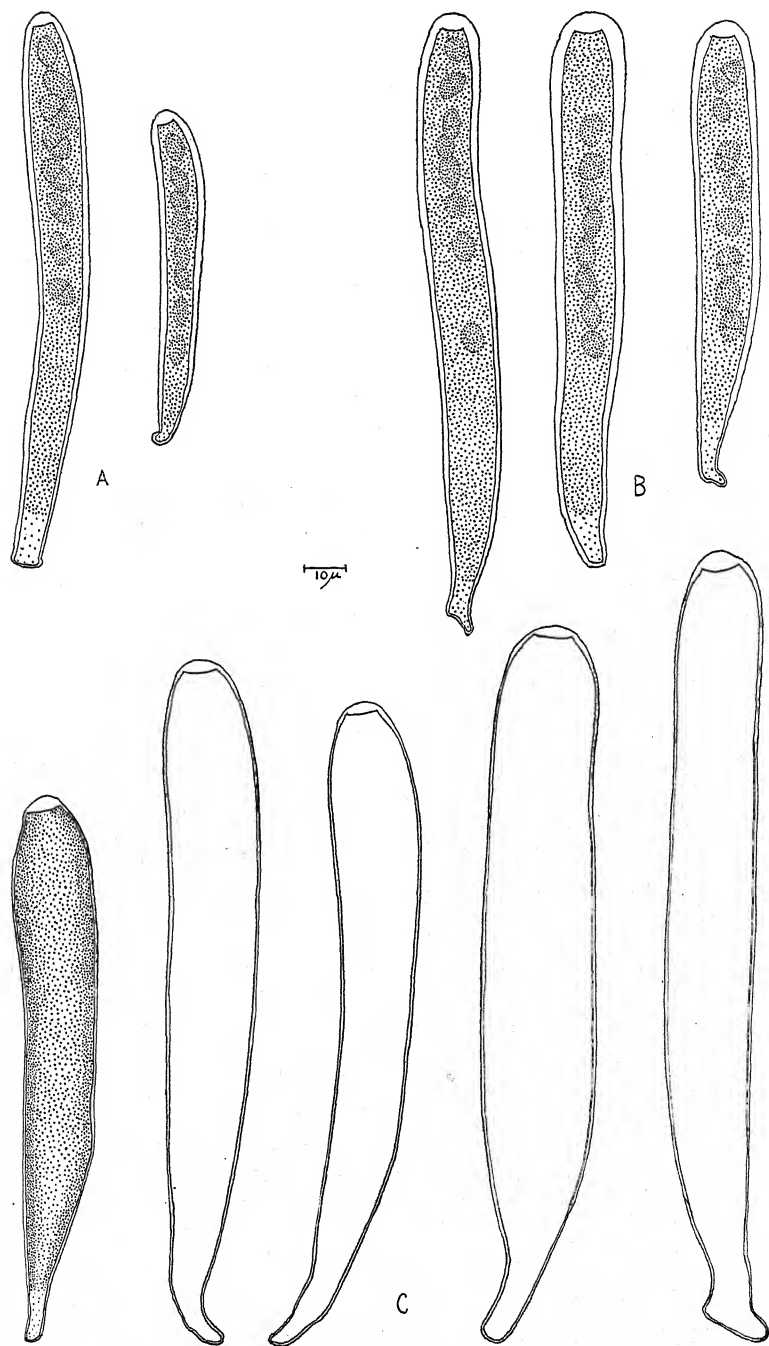
FIG. 5. Asci of *Tympanis pithya*.

celled, cylindric to allantoid, borne at the tip and along the sides of the conidiophore, 2.0–4.0 × 1.0–1.5 μ.

HOST: *Pinus strobus* L., *P. albicaulis* Engelm., *P. monticola* Dougl., *P. resinosa* Solander ex Aiton, *Pinus* spp.

EXSICCATI: Karst. Fung. Fenn. *661* Type.

SPECIMENS EXAMINED: **Canada**: Quebec: Bonaventure Co., DAOM[4] *3788;* Burnet, JWG *501;* Gatineau Park, DAOM *3828;* JWG *112;* JWG *809.* Ontario: Timagami Forest Reserve, T *3530,* JWG *39;* T *3526,* JWG *47;* T *6569,* JWG *265;* JWG *302;* JWG *481;* Charleston Lake, JWG *95;* Toronto, JWG *142;* Corkery, JWG *645.*

United States: Massachusetts: Princeton, JRH *1105,* JWG *347;* Huntingdon, JRH *1145,* JWG *367;* Topsfield, JRH *1513,* JWG *376;* Hamilton, JRH *1514,* JWG *377.* Connecticut: Hamden, JRH *1518,* JWG *379.* Vermont: Waterford, JRH *1102,* JWG *345;* Sharon, JRH *1520,* JWG *381.* New York: Lake Placid, JRH *1143,* JWG *365;* Dannemorra, JRH *1144,* JWG *366;* Canadice, JRH *1538,* JWG *388;* Alder Creek, JRH *1542,* JWG *392.* Idaho: Bonner Co., UIFP *2490,* JWG *761;* UIFP *1980B,* JWG *781.* California: Mt. Shasta, WBC *18043,* JWG *833;* WBC *20487,* JWG *867.*

TYMPANIS CONFUSA Nyl. Obs. Pez. Fenn. p. 69. 1868.

> *Tympanis amphiboloides* var. *hypopodiza* Nyl. Obs. Pez. Fenn. p. 71. 1868.
>
> *Scleroderris hypopodiza* Sacc. Syll. Fung. **8**: 597. 1889.

Apothecia erumpent, gregarious, separate or cespitose, usually with less than six in a cluster but occasionally in large clusters of fifteen or more, circular or slightly undulate, sessile to substipitate, narrowed below, 0.5–1.5 mm. in diameter, 0.4–2.0 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous-fleshy when moist; hymenium concave to plane, dull black or shiny black, more fleshy than the excipulum, at first with

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[4] The code letters refer to the following herbaria: DAOM, Mycological Herbarium of the Division of Botany and Plant Pathology, Central Experimental Farm, Ottawa; T, Mycological Herbarium of the University of Toronto; UIFP, University of Idaho, Forest Pathology Herbarium; JWG, Herbarium of J. W. Groves; JRH, Herbarium of J. R. Hansbrough; WBC, Herbarium of Wm. Bridge Cooke.
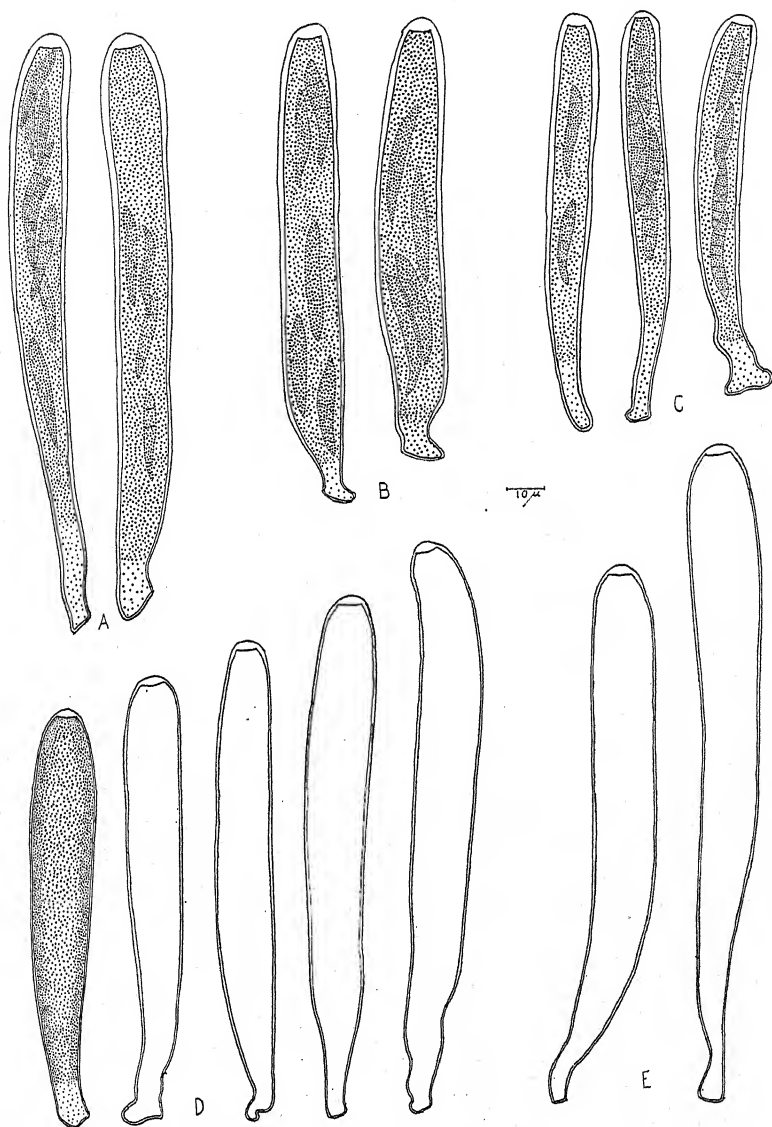
Fig. 6. Asci of *Tympanis confusa*.

a thick, somewhat infolded margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of inter-woven, ascending, brownish to nearly hyaline hyphae about 1–2 μ in diameter, with the walls greatly thickened and gelatinized, be-coming thicker and darker at the outside forming a rind-like ex-

cipulum, subhymenium not clearly differentiated; asci cylindric to cylindric-clavate, short-stalked, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first 1–4–(8)-spored, finally multi-spored, (80)–85–120–(150) × (10)–12–16–(21) μ; primary ascospores hyaline, elongate-fusoid to clavate-fusoid, one- to several-celled, occasionally muriform, irregularly biseriate to uniseriate, 13–20 × 2–4 μ; secondary ascospores hyaline, cylindric to allantoid, one-celled, 2.0–3.0 × 1.0–1.5 μ; paraphyses hyaline, filiform, septate, simple or branched, 1.5–2.0 μ in diameter, the tips slightly swollen to 3.0 μ and embedded in a gelatinous matrix forming a brownish epithecium.

Conidial fruiting bodies erumpent, scattered to gregarious, separate or cespitose, black, glabrous, globose to ovoid, sessile, 0.2–0.3 mm. in diameter, or sometimes on long, branched, cylindric or irregular stalks 0.1–0.3 mm. in diameter and up to 2.0 mm. in height, consistency similar to the apothecia; tissue plectenchymatous, similar to that of the apothecia, in the upper part containing a globose to ovoid cavity lined with conidiophores and tearing open at the top; conidiophores hyaline, filiform, septate, simple, about 25–60 × 2.0–2.5 μ; conidia hyaline, one-celled, cylindric to allantoid, borne at the tip and along the sides of the conidiophore, 2.0–4.0 × 1.0–1.5 μ.

HOST: *Pinus resinosa* Solander ex Aiton, *P. strobus* L., *P. albicaulis* Engelm., *P. monticola* Dougl., *P. contorta* Dougl., *P. sylvestris* L., *Pinus* spp.

EXSICCATI: Fries Sclerom. Suec. *336* (as *Patellaria atrata β*); Karst. Fung. Fenn. *753* (as *T. amphiboloides* var. *hypopodiza*).

SPECIMENS EXAMINED: **Canada**: Ontario: Petawawa Forest Experiment Station, JWG *688*.

United States: Connecticut: Woodbridge, JRH *1126*, JWG *359*; JRH *1134*, JWG *361*; JRH *1136*, JWG *362*; JRH *1509*, JWG *373*; Branford, JRH *1131*, JWG *360*; Windsor, JRH *1120*, JWG *357*; JRH *1108*, JWG *348*; JRH *1109*, JWG *349*; Prospect, JRH *1110*, JWG *350*; Bethany, JRH *1111*, JWG *351*; Hamden, JWG *878* (separated from JWG *379 T. pithya*). New York: Alder Creek, JRH *1544*, JWG *400*; JRH *1543*, JWG *393*; Canadice, JRH *1539*, JWG *389*; JRH *1540*, JWG *390*; Olive, JRH *1541*, JWG *391*. Idaho; Bonner Co., UIFP *2000B*, JWG *756*. Oregon: Rhododendron, JRH *1117*, JWG *355*; JRH *1118*, JWG *356*.

TYMPANIS HYPOPODIA Nyl. Obs. Pez. Fenn. p. 72. 1868.

Apothecia erumpent, gregarious, separate to cespitose with usually less than six in a cluster, circular or undulate, sessile to sub-
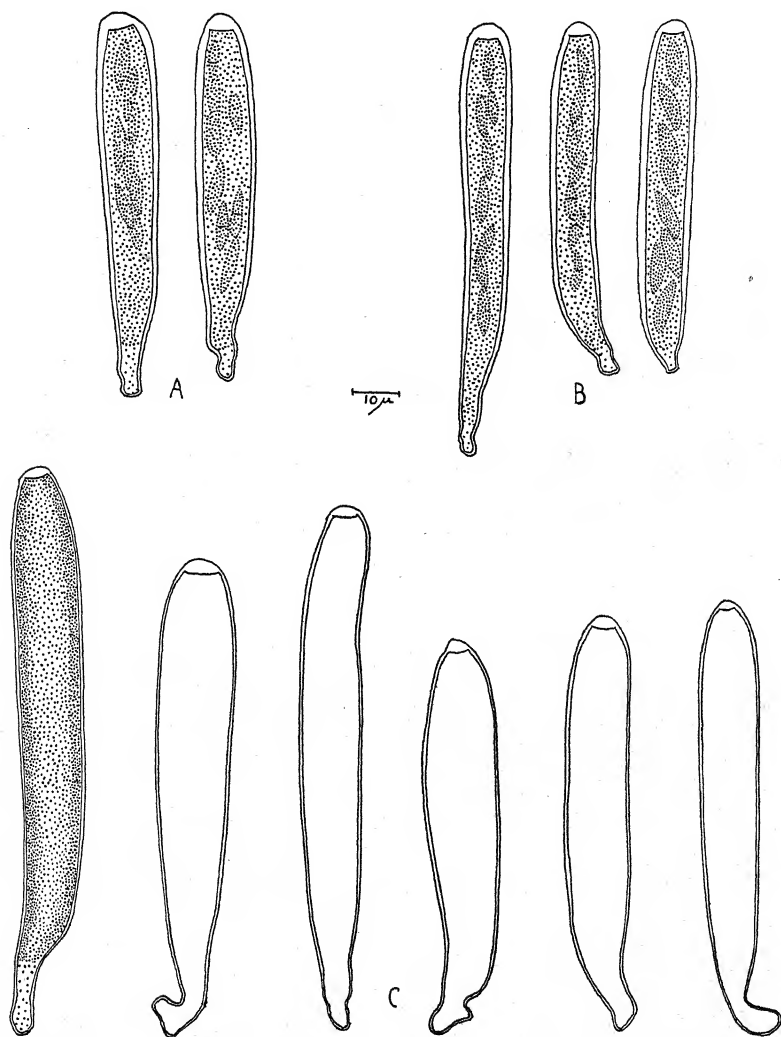
FIG. 7. Asci of *Tympanis hypopodia*.

stipitate, narrowed below, 0.5–1.0 mm. in diameter, 0.3–1.5 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous-fleshy when moist; hymenium concave to plane,

black, more fleshy than the excipulum, at first with a thick, somewhat infolded margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about $1–2\,\mu$ in diameter, with the walls greatly thickened and gelatinized, becoming thicker and darker at the outside forming a rind-like excipulum, subhymenium not clearly differentiated; asci cylindric to cylindric-clavate, short-stalked, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multi-spored, $(60)–70–90–(100) \times (8.5)–9.0–12.0–(14)\,\mu$; primary ascospores hyaline, fusiform, one- or two-celled, irregularly biseriate, $(6)–8–10–(12) \times 2.0–4.0\,\mu$; secondary ascospores hyaline, cylindric to allantoid, one-celled, $2.0–3.0 \times 1.0–1.5\,\mu$; paraphyses hyaline, filiform, septate, simple or branched, $1.5–2.0\,\mu$ in diameter, the tips slightly swollen up to $3.0\,\mu$ and embedded in a gelatinous matrix forming a brownish epithecium.

Conidial fruiting bodies erumpent, gregarious to scattered, separate or cespitose, black, glabrous, globose to ovoid or cylindric, sessile, 0.1–0.3 mm. in diameter, 0.1–0.3 mm. in height, similar in consistency to the apothecia; tissue plectenchymatous, similar to that of the apothecia, in the upper part containing a globose to ovoid cavity lined with conidiophores and tearing open at the top; conidiophores hyaline, filiform, septate, simple, $25–70 \times 2.0–2.5\,\mu$; conidia hyaline, cylindric to allantoid, one-celled, borne at the tip and along the sides of the conidiophore, $2.0–4.0 \times 1.0–1.5\,\mu$.

HOST: *Pinus strobus* L., *P. monticola* Dougl., *P. cembra* L., *P. rigida* Mill., *P. resinosa* Solander ex Aiton, *Pinus* spp.

EXSICCATI: Karst. Fung. Fenn. *754;* Rel. Farl. *156b* (as *T. Pinastri*); Rab. Fung. Eur. *1231* (as *T. Pinastri*).

SPECIMENS EXAMINED: **Canada**: Ontario: Timagami Forest Reserve, JWG *327;* JWG *300;* Petawawa Forest Experiment Station, JWG *564;* Corkery, JWG *879.*

United States: Massachusetts: Sunderland, JRH *6*, JWG *334;* Ipswich, JRH *1512*, JWG *375.* Connecticut: East Granby, JRH *1104*, JWG *346.* Idaho: Shoshone Co., UIFP *2343*, JWG *707;* Bonner Co., UIFP *2726*, JWG *763;* UIFP *1848A*, JWG *780;* Clearwater Natl. Forest, DAOM ex Herb. J. R. Weir *16615.*

mens; to Dr. R. W. G. Dennis for making specimens in Kew
Herbarium available for examination; and to Miss E. K. Cash
and the late Dr. D. H. Linder for the loan of slides of exsiccati.

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EXPLANATION OF FIGURES

FIG. 1. Apothecia of *T. confusa*. FIG. 2. Apothecia of *T. pithya*. FIG. 3.
Apothecia of *T. hypopodia*. FIG. 4. Conidial state of *T. pithya*. All × 4
approx.

FIG. 5. Drawings of asci of *T. pithya; A*, asci with primary ascospores
from Karst. Fung. Fenn. *661, type; B*, asci with primary ascospores from
North American material; *C*, five mature asci from North American
material.

Fig. 6. Drawings of asci of *T. confusa; A,* asci with primary ascospores from Fries Sclerom. Suec. *336, type; B,* asci with primary ascospores from Karst. Fung. Fenn. *753* labelled *T. amphiboloides* var. *hypopodiza; C,* asci with primary ascospores from North American material; *D,* five mature asci from North American material; *E,* two mature asci from Fries Sclerom. Suec. *336.*

Fig. 7. Drawings of asci of *T. hypopodia; A,* asci with primary ascospores from Karst. Fung. Fenn. *754; B,* asci with primary ascospores from North American material; *C,* six mature asci from North American material.

THE GENUS CERACEA CRAGIN

G. W. MARTIN

(WITH 13 FIGURES)

The genus *Ceracea* was established by Cragin (Bull. Washburn Coll. Lab. Nat. Hist. **1**: 82. 1884) on the basis of a single collection which consisted of a waxy incrustation covering a cluster of pilei of a pore fungus, referred to as "apparently *P. versicolor.*" The essential characters in Cragin's generic description were the effused, waxy habit and the "mostly bifurcate" sporophores. In the description of the type species, *C. vernicosa,* the spores are said to be borne "at the apices of the basidia." The clear inference from these statements is that *Ceracea* is a member of the Dacrymycetaceae characterized by a broadly effused, waxy basidiocarp. As such it was accepted by Patouillard (Bull. Soc. Myc. Fr. **9**: 141. 1893), who assigned to it a second species from Ecuador, by Hennings (in Engler and Prantl, Nat. Pfl. I. 1**: 99. 1897), by Bresadola (in Krieger, Fungi Sax. No. *1909*. 1906), who recorded a species from Saxony, by Bourdot and Galzin (Bull. Soc. Myc. Fr. **39**: 266. 1923), who added an additional species from France, by Killermann (in Engler and Prantl, Nat. Pfl. ed. 2. **6**: 120. 1928) and by several authors since.

Lloyd (Myc. Notes **6**: 899. 1920) stated that there is no doubt that *Ceracea* Cragin is the same as *Arrhytidia* Berk. & Curt. published many years earlier. The comment by Coker (Jour. Elisha Mitchell Soc. **43**: 237. 1928) implies that he agreed with Lloyd. Killermann cites Berkeley's genus as a synonym of *Ceracea.* Why, in such case, he did not use the earlier name is not clear, but the form of citation suggests that he may have supposed it to be a *nomen nudum.* Brasfield (Am. Midl. Nat. **20**: 213. 1938) cited *"Ceracea* auct. not Cragin" as a synonym of *Arrhytidia* but later (Lloydia **3**: 106. 1940) he recorded an Iowa collection referred to *Ceracea crustulina* Bourd. & Galz. and gave it as his opinion that the genus *Ceracea* is valid but should be "restricted to

the thin, resupinate, easily separable forms which show no evidence of distinct rooting portions." Jackson and Martin (Mycologia 32 : 693. 1940) accepted the genus on that basis and added an additional species, and, with elimination of the reference to the separable character, it was the sense in which I adopted it in the survey of the Tremellales of the north central United States (Univ. Iowa Stud. Nat. Hist. 18³: 23. 1944), restricting *Arrhytidia* to those species which are at first discoid or pustulate and rooted at the center but which early anastomose and form a more or less continuous film.

Some years ago I was informed that most of Cragin's specimens had been destroyed by fire and that the type of *C. vernicosa* was probably among them. Recently, however, Dr. D. P. Rogers has discovered in the Ellis collection at the New York Botanical Garden what is undoubtedly a substantial part if not all of the material on which Cragin's genus was based (FIG. 1) and it is therefore possible to clarify our conception of it. There are seven sporophores or clusters of a pore fungus, together with a few smaller fragments, quite probably representing *Polyporus versicolor,* as Cragin suggested. The upper surface is completely and the lower surface partly covered by a dull, reddish-brown, horny incrustation. The interior has been largely destroyed by insects but the surface has apparently been untouched (FIG. 2). When soaked, the surface layer becomes waxy and paler. Microscopic examination shows that this region is composed of densely packed, branching conidiophores (FIG. 3), the spore-bearing branchlets often more or less thickened, sometimes notably so, and the terminal branchlets often in pairs (FIG. 7), suggesting basidia of the *Dacrymyces*-type. The spores are borne profusely, much of the outer, horny portion being composed of densely agglutinated spores. They are apparently borne singly. No chains were observed and there is only one point of attachment visible on each spore. The shape is irregular; some are suballantoid and look like *Dacrymyces* spores, but the majority are ovate, tear-shaped, oblong or irregular. Empty spore-case cases suggest that in some instances the contents have slipped out. In size they are mostly 6–10 × 3–5 μ with a few spores above and below these limits.
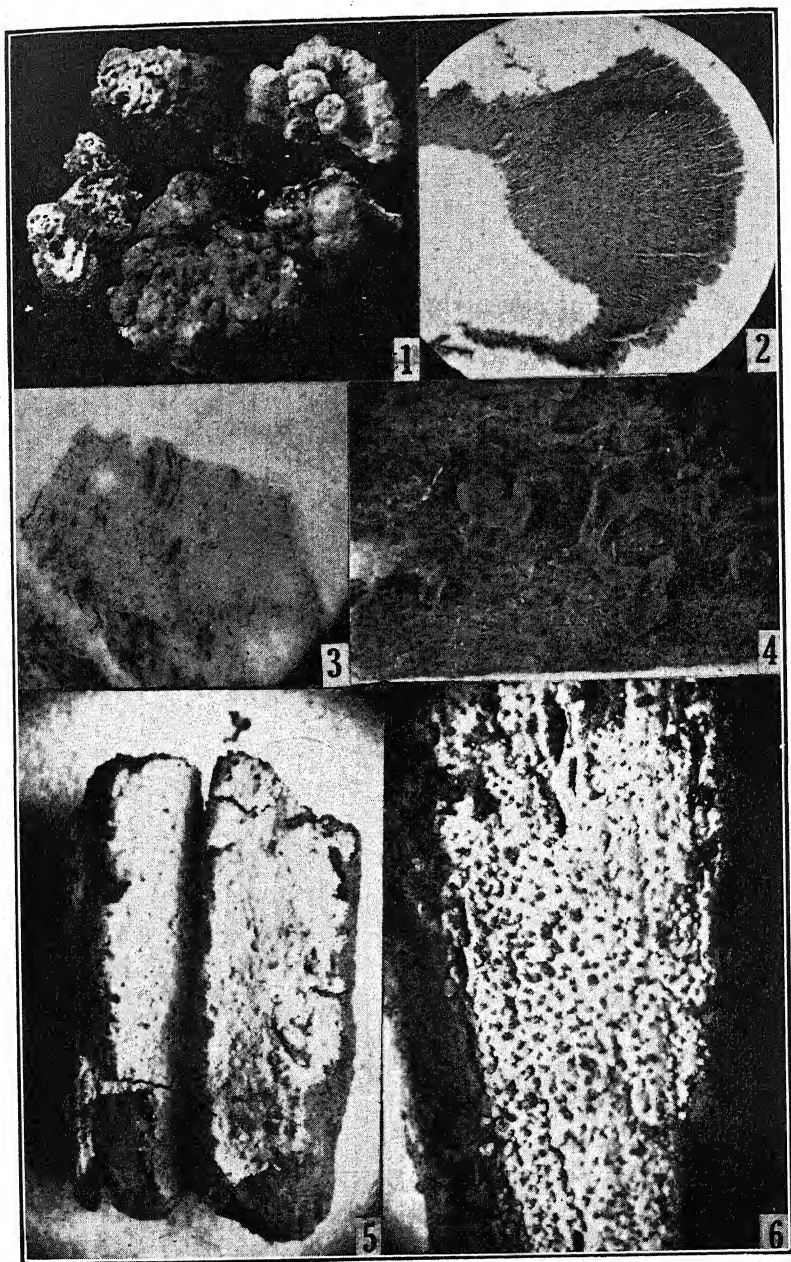
Figs. 1-6.   *Ceracea, Arrhytidia* and *Cerinomyces.*

Obviously *Ceracea* is an imperfect fungus, to be classed with the Moniliaceae, possibly near *Monosporium*. Its removal from the Dacrymycetaceae raises the question whether the broadly effused, subarid or waxy species belonging to that family and heretofore referred to *Ceracea* may properly be included in *Arrhytidia* or should have a new genus erected for them.

The original generic description of *Arrhytidia* Berk. & Curt. (Hooker's Jour. Bot. and Kew Misc. 1: 235. 1849) is as follows: "Hymenophorum a mycelio mucedineo contexto formatum marginatum, tectum hymenio ceraceo molli laevi sine plicis. Sporae oblongae." The emphasis is on the smooth, waxy hymenium with mycelioid margin. The description of the type species, *A. flava* Berk. & Curt., immediately following, adds that the species occurs on pine branches, that it forms scattered, sometimes confluent patches, that the hymenium is orange-yellow, bordered by the white mycelial margin and that the spores are obliquely attached. Berkeley summarizes his conception by comparing it with *Psilopezia* and adding: "It is a distinctly bordered, mostly pezizaeform *Merulius,* destitute of folds." Shortly thereafter Fries (Nova Acta Soc. Sci. Upsal. III. 1: 114. 1851) stated that under the microscope *Arrhytidia* had the organization of a *Dacrymyces* and that in an old fructification it could scarcely be distinguished from that genus.

Patouillard (Bull. Soc. Myc. Fr. 11: 211. 1895), in describing new species of *Guepiniopsis* from Ecuador, states of two of them that they might perhaps be referred to *Arrhytidia* because of the pezizoid form of their fructifications. These, it will be noted, are cupulate and stipitate species and the emphasis is therefore on Berkeley's use of the word pezizaeform. He adds: "ce dernier genre [*i.e., Arrhytidia*] ne présente pas de caractères tranchés le séparant de *Guepiniopsis*. *A. flava* Berk., dont nous avons pu étudier des spécimens authentiques, a un pied radicant et des dimensions plus grandes, mais tous ses autres caractères sont ceux de *G. andinus*." Further on, he emphasizes the intermediates between the genera of the Dacrymycetaceae as then recognized, but cites the "plaques corticioïdes" of *Ceracea* as representing one extreme.

Coker, in the 1928 paper cited, proposed the name *Arrhytidia involuta* (Schw.) Coker, based on *Dacrymyces involutus* Schw., reducing *A. flava* Berk. & Curt., *Dacrymyces corticioides* Ell. & Ev., *Ceracea corticioides* (Ell. & Ev.) Pat. and *Ceracea aureo-fulva* Bres. to synonymy, and was inclined to agree with Lloyd that *Ceracea Lagerheimii* Pat. is also a synonym.

Miss E. M. Wakefield has kindly examined the type of *Arrhytidia flava* at Kew and has compared it with the type of *Ceracea aureo-fulva* in the British Museum, and states: "I think they are certainly not the same species. Whether they belong to the same genus, I am not sure. . . . The spores of *Ceracea aureo-fulva* Bres. are as described, 11–$17 \times 5\frac{1}{2}$–7μ, but I was unable to see any septation in the British Museum specimen. The spores of *Arrhytidia flava* (type) are larger, 20–25×7–8μ and become 3-septate." She adds that Ravenel No. *1016* from South Carolina appears to be similar, but a specimen from Alabama, collected by Peters and named by Berkeley, has smaller spores, is obviously gelatinous and may be *Dacrymyces involutus, i.e., Arrhytidia involuta* Coker, 1928. A specimen from Coker, sent as *D. involutus*, is effused, but was obviously gelatinous when fresh and has the smaller spores.

Dr. Walter H. Snell, of Brown University, has permitted me to examine a collection from the Curtis Herbarium labelled *"Arrhytidia flava* B. & C. Ala.,*"* presumably in Curtis' hand. The specimen consists of a small rectangle of decorticated wood, about 3×1.5 cm. in size, bearing a number of flat, discoid, often anastomosing fructifications (FIG. 4). The substratum appears to be wood of a soft, frondose tree, possibly *Liriodendron* or *Populus;* it is certainly not that of a conifer. The color, dry, is about Mikado Brown (R)[1] with paler (Clay Color R) margins. The individual disks, when free, range from 1 to 2.5 mm. in diameter, each attached by a stout, root-like base. Anastomosis apparently begins rather early and causes the disks to become irregular in outline, but in no case was the junction between adjacent disks completely obliterated. The basidia are typical of the family

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[1] (R) refers to color terms as used by Ridgway, Color Standards and Nomenclature. 1912.

(FIG. 8). There may be a clamp-connection at the base of each basidium, but since the supporting hyphae collapse early, this is not clear. Clamp-connections are abundant on the internal hyphae and are of the loop type characteristic of gelatinous Basidiomycetes. The few spores seen were short-cylindrical and adaxially depressed or suballantoid, $14–16 \times 5.5–7 \mu$. No septate spores were seen. This specimen agrees with the Peters collection from Alabama as described by Miss Wakefield and essentially with Coker's description of *A. involuta* except for the absence of septate spores.

It seems clear that the specimens which have been referred to *Arrhytidia* and to *Ceracea* (exclusive of the type) fall into two groups. One group originates as pustules, each pustule rooted in the substratum, but anastomosing early and forming reticulate or more or less continuous, tough-gelatinous or subgelatinous films, drying horny. The members of the other group originate as corticioid plaques on a mycelial base, becoming broadly effused, are without conspicuous rooting bases and are waxy to subfleshy in consistency, drying arid. For the members of the first group the generic name *Arrhytidia* is available. The members of the second group are, in my opinion, generically distinct and, since *Ceracea* is not available, I propose for them the following genus:

Cerinomyces gen. nov.

Fructificationibus resupinatis, late effusis, eradicatis; hymenio leve vel tuberculoso, texto subarido vel ceraceo non gelatinoso; basidiis clavatis demum bifurcatis.

Thin, resupinate, originating as flat hymenial areas on a mycelial base, quickly becoming effused, without rooting bases, subarid to waxy, not gelatinous; hymenium smooth, spiny or tuberculate, composed of densely packed basidia and paraphyses; basidia at first cylindrical, then clavate, finally furcate by the development of two thick epibasidia, each of these tipped by a sterigma and a basidiospore; basidiospores cylindrical to allantoid, remaining simple or becoming transversely septate.

Name from κήρινος (waxy) + μύκης.

Type species:

Cerinomyces pallidus sp. nov.

Fructificatio ceracea aut aride-carnosa haud a tuberculis radicantibus orta, sed ut plagula minuta in reticulo crasso strata, inde latissime effusa, denique ultra 14 × 3 cm. effusa; hymenio vivo pallido vel isabellino, in vetustate olivaceo-fulvo, arescente pallido aut isobellino usque ad ravum; strato hymeniale levi, vel papillis
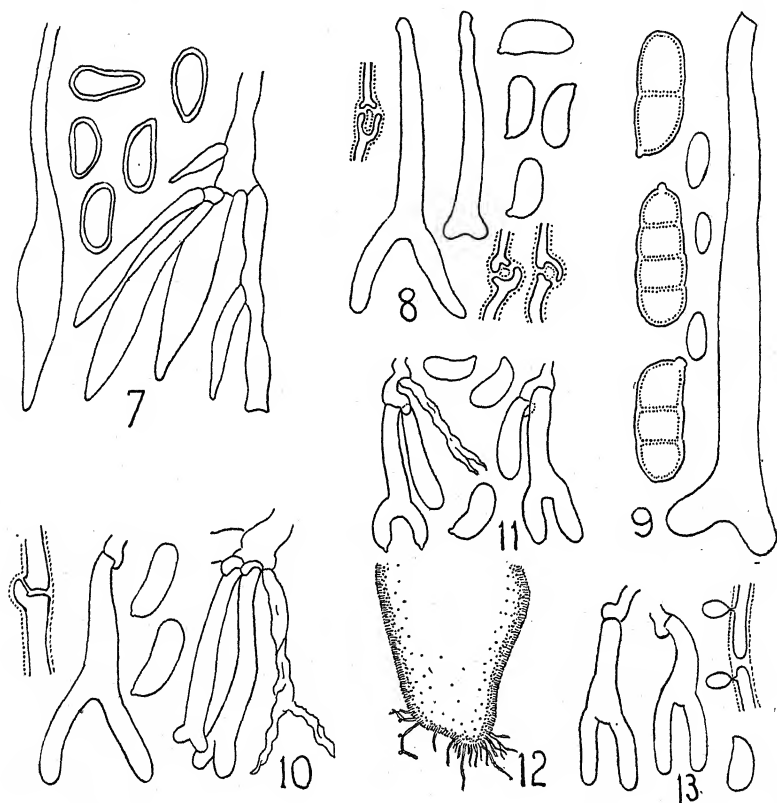
FIGS. 7–13. *Ceracea, Arrhytidia* and *Cerinomyces.*

aut aculeis parcissime inaequalibus vel subinde densissime gregariis notato; probasidiis cylindraceo-clavatis, 11–13 × 3–4 μ, duo epibasidia crassiuscula breva gerentibus; basidiis maturis apice furcatis 18–22 μ in toto longitudine; sporis cylindraceis, curvulis, 7–8 × 4–4.5 μ, integris.

Waxy to arid-fleshy, not originating as rooted tubercles, beginning as small corticioid plaques on a coarse mycelial network, becoming broadly effused, finally attaining dimensions of 14 × 3

cm. or more; hymenium, when fresh, dingy white to tilleul buff (R) or tawny olive (R) in the thicker and older portions, pallid or tilleul buff (R) to cinnamon drab (R) at margin, smooth or marked with sparsely scattered or sometimes densely clustered spines or tubercles; probasidia cylindrical-clavate, 11–13 × 3–4 μ, producing two rather short and thick epibasidia; mature basidia furcate, 18–22 μ in total length; basidiospores cylindrical, more or less curved, (6–)7–8(–9) × (3–)4–4.5 μ, remaining unseptate.

Iowa: Iowa City, on rotten oak, 28 July 1939, G. W. M. *4673*, **type**; also G. W. M. *5180*, on oak, and *3914, 3916, 5106, 5500*, on apple, all collected in Iowa City in 1939 and 1940, and from May to November.

These are the collections previously referred to *Ceracea crustulina* Bourd. & Galz. by Brasfield and myself. Through the kindness of Dr. Marcel Locquin I have been permitted to examine a portion of the type of *C. crustulina* labelled "Pl. du Gard, Galzin No. *5793*." The material seen consists of a continuous film about 8 × 2 mm. in extent, with a marginal area characterized by a coarse mycelial weft bearing scattered fruiting areas varying from less than 1 mm. across down to mere expansions at the junction of the mycelial strands. The color of the fruiting area in dry material is very close to brownish drab (R). In the continuous portion there is evidence of the fusion of separate appressed patches similar to those in the marginal area. The texture is subfleshy, not horny, and there is no reason to believe that it is at all gelatinous when soaked. The probasidia are cylindrical-clavate, 20–25 × 2–4 μ before the development of the epibasidia, becoming forked in characteristic dacrymycetaceous fashion; the basidiospores are cylindrical, slightly curved, without septa so far as observed, 10–10.5 × 3.5–4 μ. These measurements are in substantial agreement with those given in the original description. Each basidium has a clamp-connection at the base and there are numerous clamp-connections on the internal hyphae. The microscopic details are shown in figure 10.

The Iowa collections here included in *Cerinomyces pallidus* are obviously closely related to *Ceracea crustulina*, but differ in the shorter and relatively stouter basidia and smaller spores (FIGS. 11, 13), the occurrence of tubercles and spines on the older hy-

menial surface (FIGS. 5, 6, 12) and in the generally more pallid color, although this last character is probably of little significance. Some of the spines bear protruding hairs at the tip (FIG. 12) and these were observed in one instance (FIG. 13, right) to bear conidia. This may have been a response to moist conditions after collection when a spore-print was being secured.

While it would ordinarily be desirable to designate one of the older species as the type of the genus, in the present instance the abundance of material of the Iowa species, permitting wide distribution of type or authentic specimens, seems to make it desirable to designate *C. pallidus* as the type.

The following new combinations are proposed: **Cerinomyces crustulinus** (Bourd. & Galz.) comb. nov., based on *Ceracea crustulina* Bourd. & Galz. Bull. Soc. Myc. France **39**: 266, 1924 and **Cerinomyces canadensis** (Jacks. & Martin) comb. nov., based on *Ceracea canadensis* Jacks. & Martin, Mycologia **32**: 693. 1940.

The concept of *Arrhytidia* here suggested is that, except for its firm-gelatinous or waxy texture, it is similar to *Dacrymyces* in its earlier stages, beginning as a group of rooted pustular or discoid fructifications which soon anastomose and eventually form a netted or more or less continuous film on the substratum. The type is *A. flava* Berk. & Curt. *A. involuta* (Schw.) Coker is probably distinct; the difference in spore size alone between the two species is far beyond the limits ordinarily regarded as possible within a single species. *A. enata* (Berk. & Curt.) Coker and *A. pustulata* Brasf. appear to be valid. *Ceracea aureo-fulva* Bres. and *C. Lagerheimii* Pat. may also be synonyms of *A. involuta,* as Coker was inclined to believe, but as to these I should be inclined to reserve judgment. I reproduce drawings (FIG. 9) from the type of *C. Lagerheimii,* now in the Farlow Herbarium, for purposes of comparison. These were made many years ago and I unfortunately did not at the time make notes of other features, but the large, gelatinous-walled spores with thick septa and the abundant conidia suggest that the species may be distinct from *A. involuta* although closely related and to be included in the same genus. *C. elongata* Pat. (Mem. Acad. Malgache **6**: 9. 1928) from Madagascar, with very large, finally 7-septate spores, appears to be clearly distinct and should, perhaps, also be referred to *Arrhytidia*.

Cerinomyces is effused from the first, the fructification beginning as small fertile areas on a loose subiculum and spreading by anastomosis and extension. It lacks the prominent rooting bases of *Arrhytidia* and the texture is fleshy to arid, in this respect and in manner of growth and appearance suggesting *Corticium* or, when the hymenium is tubercular, *Grandinia*. *C. pallidus* is the type species, *C. crustulinus* (Bourd. & Galz.) and *C. canadensis* (Jacks. & Mart.) are the other species known.

I am indebted to Dr. Donald P. Rogers for calling my attention to the existence of the type of *Ceracea vernicosa* Cragin in the Ellis Collection and to the fact that it is an imperfect fungus, and for valuable critical suggestions; to the New York Botanical Garden for the loan of the material; to Miss E. M. Wakefield for notes on the type of *Arrhytidia flava* Berk. at Kew and for comparison of that collection with the specimen of *Ceracea aureo-fulva* Bres. in the British Museum; to Dr. Walter H. Snell and Brown University for the loan of the Alabama collection of *A. flava* in the Curtis Collection, and to Dr. Marcel Locquin for the loan of the type of *Ceracea crustulina* Bourd. & Galz.

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EXPLANATION OF FIGURES

FIGS. 1–3. *Ceracea vernicosa*. FIG. 1. Type collection, × ⅘. FIG. 2. Section through hollow pileus of *Polyporus*, covered by gelatinous crust of *Ceracea*, enlarged. FIG. 3. Detail of marginal tuft, showing closely packed mass of conidiophores and spores, greatly enlarged. FIG. 4. *Arrhytidia involuta*. Photograph of specimen in Curtis collection labelled "*Arrhytidia flava* B. & C.," × 4. FIGS. 5–6. *Cerinomyces pallidus*. FIG. 5. No. *5106*, with smooth hymenium, on apple. FIG. 6. No. *3916*, with tuberculate hymenium, on oak, × 4.

FIG. 7. *Ceracea vernicosa*. Single conidiophore, cluster of conidiophores and conidia, × 1000. FIG. 8. *Arrhytidia involuta*. Basidia, clamp-connections on internal hyphae and basidiospores, from Curtis specimen labelled *A. flava*, × 570. FIG. 9. *Ceracea Lagerheimii*. Basidium, basidiospores and conidia, from type, × 1000. FIG. 10. *Cerinomyces crustulinus*. Basidium, cluster of basidia, clamp-connection from internal hypha and basidiospores, from type of *Ceracea crustulina*, × 1000. FIGS. 11–13. *Cerinomyces pallidus*. FIG. 11. Basidia and basidiospores of No. *4673*, **Type**, on oak, × 1000. FIG. 12. Diagrammatic section through tubercle of No. *3914*, on apple, × 55. Note hymenial conidia on protruding hairs, lower left. FIG. 13. Basidia, basidiospore and hymenial conidia of same, × 1000.

NOTES AND BRIEF ARTICLES

A Note on the Genus Kuntzeomyces

(WITH 1 FIGURE)

P. Hennings (Hedwigia **36**: 246. 1897) described the mono-typic genus *Didymochlamys* and its type species *D. ustilaginoidea,* occurring on ovaries of *Rhynchospora* sp. collected by Ule in Brazil. The generic name was later changed by Saccardo (Syll. Fung. **14**: 430. 1899), apparently at Hennings' request, to *Kuntzeomyces* to avoid confusion with Hooker's Rubiaceous genus *Didymochlamya.*

Von Hoehnel (Sitzb. Akad. Wiss. Wien Math. Nat. Kl. **119**: 2–4. 1910) studied Hennings' type and provided a more com-plete description, commenting in particular on the spores with very thick, three-layered membranes or walls. He did not feel that the fungus was generically distinct from *Cintractia* and he accordingly set up the new combination *C. ustilaginoidea* (P. Henn.) Hoehn. Dietel (in Engler and Prantl, Nat. Pflanzenfam. (ed. 2) **6**: 19. 1928) discusses the fungus as *Kuntzeomyces* and illustrates the spores diagrammatically. He assigns it to the Tilletiaceae in contrast to Hennings who placed it in the Ustilagi-naceae. Until the spores have been germinated its family classi-fication remains problematical although there is no reason to be-lieve it belongs other than to the latter family. Clements and Shear (The Genera of Fungi, pp. 156 and 340. 1931) set up a new name, *Perichlamys,* for this fungus and attribute it to P. Hennings without explanation. This name does not appear to be justified.

A second collection of this unique species has recently become available for study from the Chicago Museum of Natural History. It was collected in 1922 by Macbride and Featherstone at Mito, Peru, on *Rhynchospora (Dichromena) macrochaeta.* Although the collection is scanty, there is sufficient material to demonstrate the gross characters as described by von Hoehnel and the spores

in particular are quite typical. Spores from the type collection, available for study through the courtesy of the Farlow Herbarium, are illustrated in figure 1 *B* and those from the Peruvian specimen
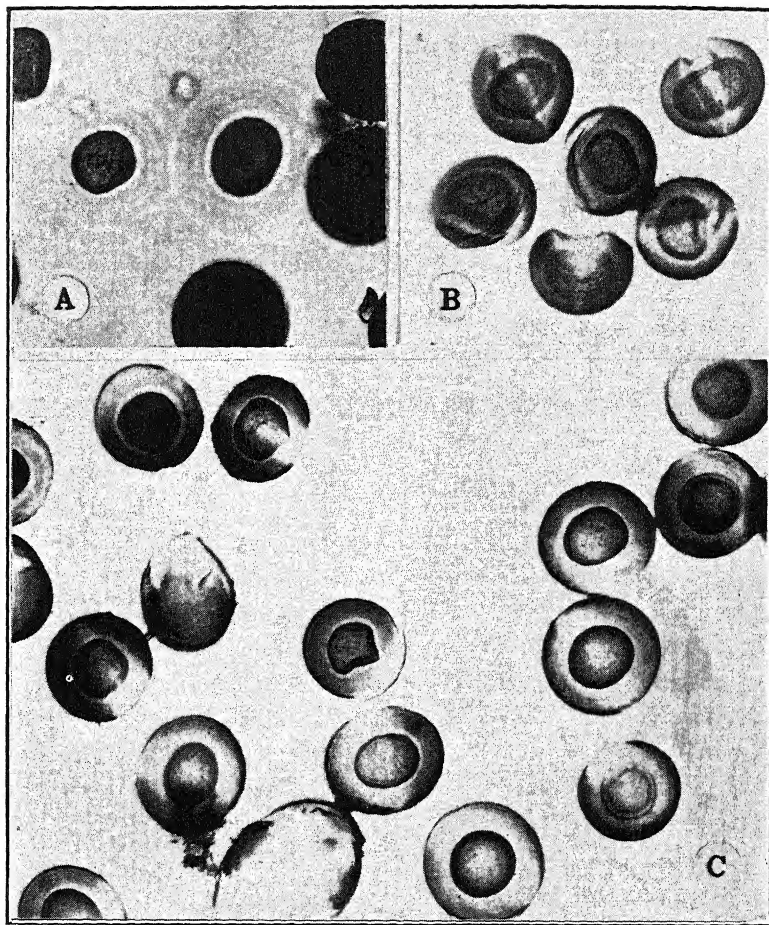
Fig. 1. *Cintractia ustilaginoidea.* *A,* spores from the Peruvian specimen, showing middle gelatinous layer; *B,* spores from the type collection; *C,* spores from the Peruvian specimen, showing outer and inner spore walls. All × 500. Photographs by A. G. Johnson.

in figure 1 *C.* The similarity of the two is striking. An attempt is made in figure 1 *A* to illustrate the gelatinous middle layer of spores from which the outer wall has broken away.

As pointed out by von Hoehnel practically the only difference between the fungus under discussion and certain species of *Cintractia,* such as *C. leucoderma* and *C. pachyderma,* lies in the possession of three layered spore membranes by the former. The outer membrane ruptures readily, permitting the spore proper with its gelatinous layer to pass out freely. Pressure of the cover slip in an ordinary mount breaks a considerable portion of the outer spore walls as seen in figure 1 *A.* As far as known this phenomenon does not take place in other species assigned to *Cintractia.* Von Hoehnel did not think these spore characters were of generic significance but the writers feel that they are and therefore prefer to maintain the fungus under the name *Kuntzeomyces ustilaginoideus* P. Henn.—Lee Ling and John A. Stevenson—(Food and Agricultural Organization of the United Nations, and Plant Industry Station, Beltsville, Md.).

Article 64 and the Nomenclature of Lichens

It is perhaps the opinion of botanists generally who have given thought to Article 64 of the International Rules of Botanical Nomenclature that the primary intent of that Article is to eliminate as a *nomen confusum* any epithet based upon a type specimen containing parts of different species which had been assumed by the author of that epithet to be one species only, as in the case of a type consisting of flowers of one species, leaves of another, etc. In instances of this kind there is proper doubt as to which of the several species the epithet may apply. Since in the case of lichens every type specimen inevitably would contain a fungus and its algal symbiont, some interpretations of Article 64 would invalidate practically all lichen nomenclature—a result most certainly not contemplated by the authors of the Article and one that actually reduces the Rule to absurdity. Such legalistic hairsplitting is, however, the inevitable result of acceptance of the widely held doctrine that a lichen is a 'dual organism,' part fungus, part alga.

It is, of course, true that most elementary text-book definitions, and those in dictionaries also, continue to propagate the notion that a lichen is a combination of two components, a fungus and an alga, living in some sort of symbiotic relationship. This con-

cept of lichens as an ecological association would logically preclude their recognition as phylogenetic and taxonomic entities by the usual system of classification; and to accept it would require a new lichen nomenclature—an entirely needless and disorganizing performance. The lichens have received unusually competent monographic treatments with a well-recognized and accepted nomenclature; in fact in the entire history of systematic botany the nomenclature of lichens has been characterized by less confusion than that of other thallophytes. It is usually taken for granted nowadays that certain erroneous concepts of a former time can be corrected without invalidating all the previous pertinent nomenclature. Thus what is commonly called a 'lichen thallus' is recognized today as a peculiar colonial development of a fungus that is in close association, symbiotic or parasitic, with a colony of algal cells, of one or more species, which it encompasses. The morphological features most significant in the taxonomy of lichens are structures clearly recognized as fungal; and the fungus in many instances can be propagated in pure culture free from its algal associate. The characters and identities of the algal 'symbionts' or 'hosts' are of secondary taxonomic significance; in fact in some cases lichen species may have more than one species of algal associates. Even if some taxonomists of lichens have recognized that in certain genera all the species are limited to one genus of algal associates that fact is of no more taxonomic consequence than recognition of the fact that in certain genera of parasitic fungi all the species are restricted to related host plants. The significance of the alga on the morphology and taxonomy of the lichen is of no more moment than that of the symbionts, hosts or nutritive substrata of many other fungi. The fact, of late widely recognized, that species of *Septobasidium* derive their nutrition primarily from scale-insects rather than from the bark of trees to which they are attached, and that this relation is responsible in large part for their peculiar lichen-like aspect has caused no fear that the nomenclature of species of *Septobasidium* is invalid. Likewise recognition of the symbiotic relation of certain agarics and boletes to the roots of some higher plants can hardly be expected to have any revolutionary effect upon the nomenclature of either associate. If later investigations disclos-

ing new information on the nutritive relations of an organism necessarily invalidates its name then all nomenclature becomes an irrational nightmare.

The fact that original descriptions of lichens have included characters of the gonidia (cells of the algal associate), unfortunately often called the "algal component of the lichen," is small cause for invalidating their names. Recognition of the relation of gonidia adds no real confusion to lichen systematics. Most descriptions of plants as originally drawn later require emendation; and the fact that most descriptions of lichens assumed or stated that gonidia were an integral part of the named organism rather than symbionts or hosts, as is the modern concept, is fundamentally inconsequential, except for the assumption in some quarters that these earlier descriptions contravened Article 64 of the present-day Rules.

The modern concept of lichens with gonidia recognized as symbionts or hosts should preclude the invalidation of their names on account of the wording of Article 64; but, lest the present wording of that Article provide any impediment to the present well accepted nomenclature of lichens, in the minds of those who prefer to regard a lichen as a 'dual organism' it is here proposed that it be emended as follows:

Art. 64.—add sentence to read as follows:

"For purposes of nomenclature lichens are to be treated as fungi except as limited by Art. 20 (d)."

—WILLIAM W. DIEHL, U. S. Plant Industry Station,
Beltsville, Maryland.

SOME XYLARIAS FROM PANAMA [1]

For about sixteen months in 1944–45, the writer was on duty at the Army School of Malariology in the Panama Canal Zone. During that period there were only occasional opportunities for collecting plants. Among the fungi collected at that time were eight species in the genus *Xylaria* Hill. Determinations were made by Dr. Julian H. Miller and dried specimens were placed

[1] Contributions from the Botanical Laboratory, The University of Tennessee, N. Ser. No. 104.

in the herbarium of The University of Tennessee. It is believed that these collections should be recorded here.

Xylaria cubensis Mont. No. 16598, on decaying wood, near La Joya, Republic of Panama, July 18, 1944. Listed from Barro Colorado Island in the Canal Zone by Standley (Smith. Misc. Coll., **78**, No. 8, 1–32, 1927).

Xylaria rhizomorpha Mont. No. 16600, on decaying wood, near La Joya, Republic of Panama, July 18, 1944.

Xylaria scruposa Mont. No. 16604, on log, Barro Colorado Island, Canal Zone, July 21, 1944.

Xylaria comosa Mont. No. 16605, on decaying wood, Barro Colorado Island, Canal Zone, July 21, 1944. Reported by Weston (Sci. Monthly, **36**: 387–407, 1933) from Barro Colorado Island.

Xylaria grammica Mont. No. 16621, on log, near La Joya, Republic of Panama, Oct. 12, 1944. Reported by Weston (Sci. Monthly, **36**: 387–407, 1933) from Barro Colorado Island.

Xylaria guayanensis Mont. No. 16625, on log, near La Joya, Republic of Panama, Oct. 12, 1944.

Xylaria scopiformis Mont. No. 16626, on log, near La Joya, Republic of Panama, Oct. 12, 1944.

Xylaria multiplex (Kze. ex Fr.) B. & C. No. 16631, on log, Barro Colorado Island, Canal Zone, Nov. 5, 1944.—SAMUEL L. MEYER.

SCIENTISTS AND RESERVE OFFICERS

The Department of the Army has established a program of particular interest to mycologists and other scientists who hold Reserve commissions in the Army, and who are professionally engaged in teaching or research and development.

The objectives of the program are to:

(1) maintain the useful affiliation of mycologists and other scientists with the Organized Reserve Corps,
(2) provide peacetime Reserve assignments for these officers, enabling optimum utilization of their education, experience and skills,
(3) furnish mobilization assignments which will fully utilize their talents, and
(4) adequately prepare these officers for mobilization.

The Technical Services of the Department of the Army submit to these Research and Development Reserve Groups research problems and projects which pose an intellectual challenge to members of the groups. Thus, the program provides members of each group a type of training which is in keeping with their scientific and technical interests and competence, rather than a traditional kind of training session in which scientists have little or no interest.

The program is now being implemented only in those areas where there is a definite local interest. To date, eighteen Research and Development Reserve groups have been organized. Twelve additional groups are in process of organization. Others are in the initial stages of formation. Several of these groups have been formed in communities in which large universities, industrial research laboratories, or private research foundations are located. Typical localities are Chicago, Illinois; Wilmington, Delaware; Newark, New Jersey; Houston, Texas; Washington, D. C.; Manhattan and Lawrence, Kansas; Champaign-Urbana, Illinois; Pittsburgh, Pennsylvania; Denver, Colorado; and Detroit, Michigan.

Provision is made to submit research projects of interest to all categories of scientists—chemists, physicists, engineers, geologists, geographers, psychologists, mathematicians, and all of the biological scientists which will include mycologists.

Reserve officers who are currently engaged in civilian research, college or university teaching, or industrial research or development, or who in the past have had specific research experience are eligible to make application for assignment to an Organized Reserve Research and Development Group. A group may be organized in any locality where there are twenty (20) or more qualified officer scientists who desire to participate in the program. A subgroup may be organized with ten (10) qualified members.

The program is under the general direction of the Research and Development Group, Logistics Division, General Staff, United States Army. The entire program is outlined in the Department of the Army Circular Number 127, dated 5 May 1948.

Inquiry about organization of an Organized Reserve Research and Development Group or about assignment to a group already

organized should be made of the Unit Instructor, ORC, or of the Senior Army Instructor, ORC, in the locality in which the officer resides. In localities in which a group has already been organized, the Commanding Officer of the group will consider applications for assignment of additional officers.

PREVENTION OF DETERIORATION ABSTRACTS

The National Research Council of the National Academy of Sciences (Prevention of Deterioration Center, Room 204), 2101 Constitution Avenue, Washington, D. C., offers the 'Prevention of Deterioration Abstracts' on a yearly subscription basis. These Abstracts are classified under the following headings: Biological Agents; Electrical and Electronic Equipment; Fungicides and Other Toxic Compounds; Lacquers, Paints and Varnishes; Leather; Lubricants; Metals; Miscellaneous; Optical Instruments and Photographic Equipment; Packaging and Storage; Plastics, Resins, Rubbers, and Waxes; Textiles and Cordage; Wood and Paper. Cross references are included in each issue; author and subject indexes are compiled at the conclusion of each volume. Material for the Abstracts is obtained from journal articles, patents, and unpublished reports from government, university, and industrial research groups both here and abroad.

Approximately 2000 pages are published a year, in two volumes of six issues each. The individual abstracts are in loose leaf form so that they may be arranged in any manner desired by the individual receiving them. Comments are added to many of the abstracts to correlate relevant information, to evaluate reports, or to make suggestions for further research.

The yearly subscription rate, which includes two sturdy binders and index guides, is currently $37.50. The rate will be $50.00 for requests received after July 1, 1949. All subscriptions run from July 1st to June 30th. Back issues are available from April 1946 when the series was started.

An 'Advance List,' a monthly bibliography of all the reports received in this field by the Prevention of Deterioration Center is also available for an additional $10.00 per year.

BOOK REVIEWS

International Rules of Botanical Nomenclature, compiled from various sources by W. H. Camp, H. W. Rickett, and C. A. Weatherby. Published in 1948 by Chronica Botanica Co., Waltham, Mass., and Stechert-Hafner, Inc., New York City. (Price $3.50.)

This publication was prepared by the Committee on Nomenclature of the American Society of Plant Taxonomists consisting of W. H. Camp, H. W. Rickett and C. A. Weatherby and was originally published in Brittonia (6: 1–120. 1947). The republication by Chronica Botanica has made the compilation generally available. The third edition of the International Rules of Botanical Nomenclature (1935) has been closely followed, with such modifications and additions as are required by the actions of the Congress at Amsterdam in 1935. One of the most important deviations from the third edition is the treatment of "Nomina Generica Conservanda." Generic names proposed for conservation have been included with officially adopted names in Appendix III. The genera of Phanerogamae are arranged according to Dalla Torre and Harms, *Gen. Siphonogam.* and the genera of other groups alphabetically. A grouping of names by families is also given for the Algae and Fungi. A general alphabetical index to all the names treated in Appendix III is added. This treatment is very useful but the inclusion of both adopted and proposed names may lead to some confusion.

It is stated in the preface that the publication is an unofficial compilation prepared to facilitate the study of nomenclatural questions. It is of special importance now when proposals for the next International Botanical Congress are under consideration.—E. B. Mains.

Biology of Pathogenic Fungi, with foreword by J. G. Hopkins, M.D. Edited by Walter J. Nickerson. Pp. i–xx, 1–236. Waltham, Mass.: The Chronica Botanica Co.; New York City: Stechert-Hafner, Inc. (Price $5.00.)

This volume is the sixth in the series Annales Cryptogamici et Phytopathologici and is in the form of a symposium of articles by various investigators. A brief introduction by the editor is followed by four papers or chapters which summarize the information available on individual fungous disease groups, each written by a specialist in the group concerned. These include accounts of the pathogenic Torulopsidaceae by J. Lodder and A. de Minjer; Chromoblastomycosis, by A. L. Carrion and M. Silva; *Pityrosporum ovale* by R. W. Benham, and of *Coccidioides* by C. W. Emmons. These accounts bring to a focus a vast amount of scattered literature, and similar accounts of other fungus pathogens would be welcome. The use of the form genus name *Fonsecaea* for the pathogens of Chromoblastomycosis which have three types of conidial formation, each of which already bears a form genus name (*Phialophora, Hormodendron, Acrotheca*), raises the question as to whether we are now liable to be inflicted with new generic names for all combinations and permutations of the conidial stages of pleomorphic fungi. The recognition and establishment of some rules of usage for form genera names, in the International Code of Nomenclature, is a matter that should receive attention.

Cifferi and Raedelli outline the recent work done in Italy in Mycophthology, and D. S. Martin discusses the geographic distribution of systemic fungous diseases, with the aid of world maps for each disease group. The remaining chapters deal with the biology of the pathogenic fungi in general. They include discussions of the action of sulfonamides and antibiotics, by F. T. Wolf; the lipids of fungi, by R. L. Peck; Nutrition and Metabolism, by W. J. Nickerson and J. W. Williams. These chapters, again bring together and synthesize much scattered information concerning the physiology of these fungi.

This volume is of particular value to those interested in the pathogenic fungi because of the biological, rather than the clinical or taxonomic, approach found in most other books on this subject. This viewpoint results in the collection and analysis of the known information on the metabolism of these fungi, which will, of necessity, be fundamental to further studies and practical applications in this field.—L. E. Wehmeyer.

CORRECTIONS

In the description of *Helicogloea Sebacinoidea* Olive (Mycologia **40**: 588–590. 1948) the basidiospore measurements should read: 5.2–8.7 × 17.4–25.2 μ.—Lindsay S. Olive.

The rust genus *Kernia* was described by the writer to accommodate a rust species on *Litsea* sp. in South India (Mycologia **38**: 679–686. 1946). In checking through the list of genera of fungi so far known, the writer found that the name *Kernia* had already been used by Nieuwland (Amer. Midl. Nat. **6**: 379–386. 1916) by transferring *Magnusia* Sacc. Neither Clements and Shear (The Genera of Fungi, 1931) nor Ainsworth and Bisby (A Dictionary of the Fungi, 1945) mention about *Kernia* Nieuwland. Since *Kernia* Nieuwland is already represented in literature, the writer proposes the name *Kernella* Thirum. for the rust on *Litsea* sp. with the type species *Kernella Lauricola* Thirum. nom. nov.—M. J. Thirumalachar.
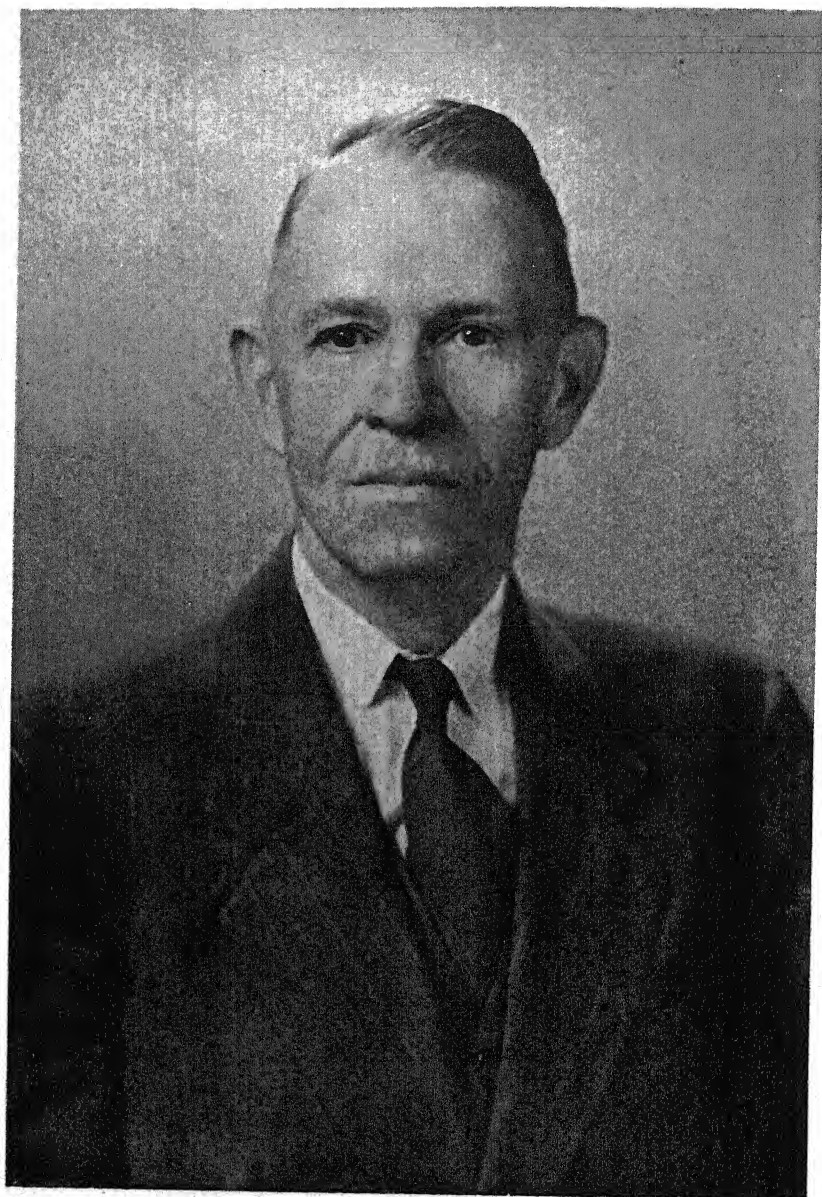
Julian H. Miller, President 1948

MYCOLOGIA

A REVISION OF THE CLASSIFICATION OF THE ASCOMYCETES WITH SPECIAL EMPHASIS ON THE PYRENO- MYCETES [1]

JULIAN H. MILLER [*]

(WITH 37 FIGURES)

Identifications in this, the largest class of the Fungi, have always been very difficult for all except specialists in small groups. Taxonomic units have been erected on gross morphological characters which fluctuate with varying environments or ecological habitats. Important fundamental characters such as those of the ascus, or those of the ascocarp centrum, have been largely ignored in establishing relationships. This has resulted in artificial groupings and keys of little practical use especially in the identification of random collections.

Since the turn of the century there have been very few comprehensive monographs of the Ascomycetes, and so the mycologist still has to fall back on one of two systems: that of Saccardo (22) in the Sylloge Fungorum, or the one in Engler und Prantl (6), Natürlichen Pflanzenfamilien. This is in spite of a wealth of discovery of more fundamental, and therefore more constant characters than those used in the above volumes.

In this paper the writer has attempted groupings based primarily on characters of the ascus correlated with ascocarp centrum char-

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[1] Lichens are not treated in this paper.
[*] Presidential address given at the Washington meeting, 1948.

acters. It is believed that this will result in a more natural system as well as in keys that can be used by the student as well as the expert.

With the publication of the Pflanzenfamilien in 1897, a classification of the Ascomycetes was presented which has been the one generally taught in American mycology courses. This has been due chiefly to the widespread use of the two books by F. L. Stevens (25, 26), which are for the most part translations of the above.

From the modern point of view the orders or suborders are apparently collections of unrelated forms. Many changes in the classification have been suggested, but so far they have not been incorporated into an over all system.

The Helvellineae, Pezizineae and Phacidiineae have been revised on the character of the ascus opening. Seaver (24) has monographed the Operculates, and Nannfeldt (19) the Inoperculates in part.

In the Hysteriineae, Nannfeldt (l.c.) has taken the Hypodermataceae out of the order and placed the members in the Helotiales, and has made the Ostropaceae the type of a new order, the Ostropales. In addition he has placed the Hysteriaceae, sensu stricto, in the Pseudosphaeriales.

The Perisporiales have also been broken up, and the Microthyriaceae placed in the Hemisphaeriales by Theissen and Sydow (28). They include in the Perisporiales only the Erysiphaceae and Perisporiaceae. The recent viewpoint is to consider the Erysiphaceae quite distinct and to erect the order Erysiphales. The concept of *Meliola* Fr. and related genera cannot be satisfactorily fixed in the system until more species have been investigated.

The Hypocreales, separated on color and texture alone, have long been recognized as an artificial assemblage. The scolecosporous forms with the ascus cap have been made the basis of another order by Nannfeldt (l.c.), the Clavicipitales.

The other large groups represented by species of *Nectria* Fr., *Hypocrea* Fr., and *Hypomyces* (Fr.) Tul. and their apparent relatives, have not been studied sufficiently from the standpoint of their

developmental histories to warrant either splitting them into families or placing them with any other Pyrenomycetes.

The Dothideales and Sphaeriales are also artificial groupings. Species in which the locules lack separate walls and in which more than one occurs in a stroma were placed in the former. If perithecia with walls are produced, the species were placed in the latter. If only one locule is present in a stroma, by interpretation the species was placed in the Sphaeriales because this could not be distinguished from a single perithecium with a wall.

Von Höhnel (9, 10) first focussed attention on the centrum or "nucleus" characters, and established the pseudosphaeriacous centrum as opposed to the sphaeriacous centrum. The former was distinguished by each ascus being in a separate locule formed of interthecial stromal threads. In the Sphaeriales one finds free paraphyses and no interthecial threads. This question has been discussed at length by von Höhnel (l.c.) Theissen and Sydow (29), Petrak (21), Nannfeldt (19), and the writer (16, 17, 18).

In Saccardo's Sylloge Fungorum the limits of the families correspond in large part to those of the orders or suborders, or in some cases to the families, of the Engler und Prantl system. The chief differences lie in the primary separations being based on spore characters in the former as opposed to position of the ascocarp relative to the substrate, amount of stroma, and other such artificial characters in the latter. Of the two the Saccardo system seems to bring together more closely related forms than the other system. However, the major groupings are open to the same criticism in both.

PRESENT REVISION

The writer proposes suggestions for a revision of the fungi previously placed in the Sphaeriales, the Dothideales, and the Hypocreales, and demonstrates the changes in a key at the end of this paper. His formerly expressed ideas as well as some of those of von Höhnel, Theissen and Sydow, Nannfeldt and others are incorporated in these changes. In addition there are a few suggestions for rearrangements in other orders. However, only separations of orders and families are attempted, as it would now be

impossible to establish a complete revision, especially down to species. This awaits the work of specialists in the groups.

Criteria of relationship are based on characters of the ontogeny, as well as of the mature apothecial or perithecial centrum, and on morphological characters of the ascus and ascospores. Such characters as position of the ascocarp relative to the substrate, or color or extent of stroma, are not considered fundamental and are ignored in the keys.

Primitiveness is thought to consist of globose to oblong or widely clavate asci, usually with a uniformly thickened wall, and opening by splitting or gelatinization of the outer wall and elongation of the inner, which in turn dissolves, freeing the spores. Also, the asci are variously scattered on mycelium or rather irregularly grouped in an ascocarp. Lastly, the asci arise following a union of an archicarp with an antheridial hypha.

The high point in development would be exhibited by cylindrical asci closely compacted in an ascocarp, usually with thin lateral walls, much thickened at the apex, and opening by a pore or lid. The asci here arise from a much branched ascogenous hyphal system, following the union of free spermatia with the trichogyne of an archicarp. The idea that spermatia and archicarps are present, and then later ascogenous hyphae arise apogamously from basal vegetative cells, could well have resulted from imperfect cytological technique. In many cases it is practically impossible to trace the connections between the asci and the archicarp with the usual paraffin section method, but that doesn't justify the conclusion of apogamy.

The writer has recognized these types of asci and ascocarp centra.

1. The globose or broadly clavate asci without a pore lie at irregular levels throughout loose or pseudoparenchymatous tissue composed of ascogenous hyphae. As evolution proceeds the asci are brought into one locule and each ascus grows up into the stroma. The monascal locule partitions are then the remnant stromal elements. These tendencies are exhibited in the Plectomycetes.
2. The clavate asci with no pore arise in a basal fascicle and in growing upward dissolve out a stromal locule. These are

multi-ascal locules, with no interthecial threads. This is the central tendency of the Dothideales.

3. The clavate to cylindrical asci with no pore arise from an entire wall-layer in a stroma and grow upward between filiform threads, which are connected at the top and bottom of the locule. Following the presence of the archicarp there is a fan-shaped array of deeply staining threads that grow downward toward the base of the locule, ultimately appearing as a palisade of vertical threads. Then the asci arise from a position at their bases and grow up between them. These threads are present before the asci arise and are here designated as pseudoparaphyses. They are characteristic of the Pseudosphaeriales, Microthyriales, Hysteriales, and Lophiostomatales.

4. The clavate to cylindrical asci with a pore arise between apically free threads, which usually precede them in development. This is the writer's concept of paraphyses. The ascocarp wall is a special structure developed from the basal cells of the archicarp and is not part of the stroma. This type of centrum is characteristic of the Sphaeriales and the Discomycetes.

3. This type has been discovered by Luttrell (15) in *Sphaerostilbe aurantiicola* of the Hypocreales. There is a true wall to the perithecium and periphyses in the ostiole, but vertical threads or pseudoparaphyses precede the asci as in the Pseudosphaeriales. The asci are as in type 4. This is a combination of types 3 and 4 in part. Should this prove characteristic of many of the species of the Hypocreales it would provide a 5th trend in Ascomycete development.

HEMIASCOMYCETES

Endomycetales and Exoascales

These follow the usual pattern and apparently represent primitiveness as well as simplicity. Nannfeldt (l.c.) leaves the Exoascales out of his key because of uncertain position, and places the Endomycetales coordinate with the Hemiasci (Dipodascaceae). The writer uses the same ordinal limits as Wolf and Wolf (32). *Taphrina caerulescens* (Mont. & Desm.) Tul. shows the characters of the ascus (FIG. 1).
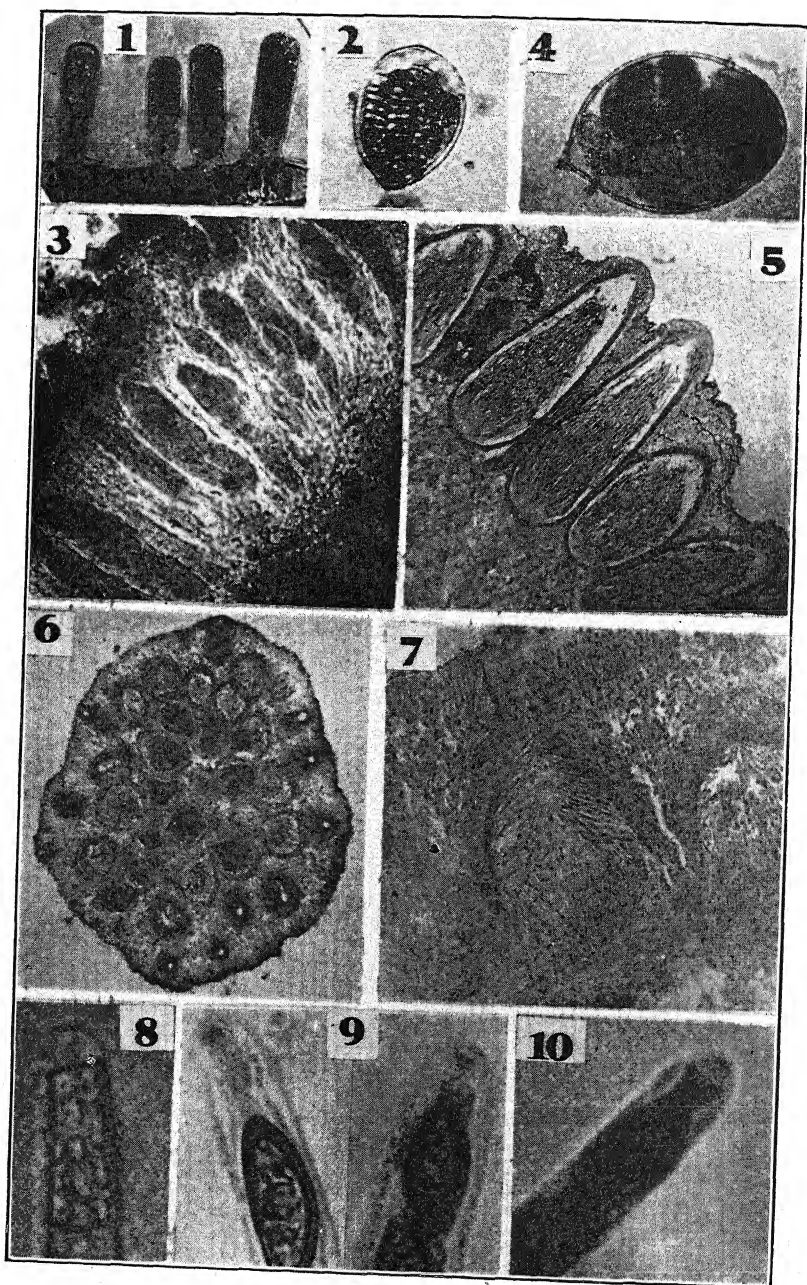
FIGS. 1–10. Types of spores, asci and ascocarps in the Ascomycetes.

EUASCOMYCETES

The old concept of asci in ascocarps is retained. Nannfeldt (l.c.) divided this subclass into 1. Plectascales, 2. Ascoloculares, and 3. Ascohymeniales. His Plectascales contain most of the same groups as are found in the Plectascineae of the Pflanzen-familien or the Plectascales of Gwynne-Vaughan and Barnes (8) or Bessey (1) (sub Aspergillales), or Wolf and Wolf (l.c.) (sub Eurotiales). His Ascoloculares and Ascohymeniales, however, are new groupings. The former comprises all of the Pyrenomycetes with asci in locules, including even the Myriangiales and Hysteriales. The Ascohymeniales on the other hand include forms with a hymenium of asci and apically free paraphyses.

The writer thinks it preferable to keep the old separations of Plectomycetes, Pyrenomycetes and Discomycetes with some modifications. For example, in Nannfeldt's Ascoloculares there are at least three ontogenetically different ascal locules, or the writer's types 1, 2 and 3. In the Myriangiales the pseudoparenchymatous locule tissue is composed of ascogenous hyphae and the asci tend to be globose which places that order in the Plectomycetes. Then in Nannfeldt's Pseudosphaeriales he has the *Pleospora*-type with connected threads between the asci, and the *Dothidea*-type with no threads. Neither von Höhnel nor Theissen and Sydow distinguished between the monascus locule composed of the vertical threads that are in the centrum before the asci appear, and the remnants of stromal elements due to the asci pushing up in the stroma as in the higher Myriangiales, such as in *Dothiora subtropica* (Wint.) Mill. & Burt. (FIG. 3).

It appears more logical to retain the term Discomycete for the Ascohymenial forms with exposed hymenium and Pyrenomycete for those with inclosed asci. Then all of Nannfeldt's forms in his Ascohymeniales with true perithecia should go in either the Sphaeriales or Hypocreales. In his Ascoloculares forms with the *Dothidea-Mycosphaerella* type of development should be placed in the Dothideales, and those with the pseudosphaeriacous type in the Pseudosphaeriales, Lophiostomatales, Hysteriales, or Microthyriales.

PLECTOMYCETES

Eurotiales, Myriangiales and Erysiphales

These are connected by the globose to semiglobose asci generally with uniformly thickened walls opening by the outer wall splitting, and ascocarps with no paraphyses or other threads and with no definite manner of opening (FIG. 2. *Myriangium Duriaei* Mont. & Berk. and FIG. 4. *Microsphaera Alni* DC. ex Wint.). The irregular arrangement of the asci, surrounded by ascogenous hyphae, and the liberation of spores within the ascocarp are characteristic except in the Erysiphales. In the latter the asci are typical, but in basal tufts.

Nannfeldt (l.c.) added the Ophiostomataceae (Ceratostomataceae) and Chaetomiaceae to his Plectascales because of asci with walls that deliquesce very early and liberate masses of spores within the ascocarp. There are no interthecial threads. This seems logical, but both groups are exceptions in possessing definite ostioles. The asci of the Plectomycetes are usually formed on the ends of hyphae or in chains, and ascus hooks are very rarely present.

The name Perisporiales is dropped because the order as delimited by Lindau (6) in the Pflanzenfamilien comprises forms that are now known to belong in several other orders. The most definite and clear-cut group with well-known life histories is the Erysiphaceae, so the name is changed to the Erysiphales as is done by Gwynne-Vaughan and Barnes (8). Not enough is known of the developmental characters of the Meliolaceae to indicate their affinities. According to Graff (7) there is an outer stroma beneath which the perithecium is hidden, with paraphyses and periphyses in an ostiole; however there is an oogonial and antheridial stalk. The sex organs are similar to those of the Erysiphaceae, but the centrum characters are apparently those of the Sphaeriales.

DISCOMYCETES

This subclass includes species with a hymenium composed of asci with a pore or lid and apically free paraphyses, exposed at least at maturity. This comprises the Discomycete part of Nann-
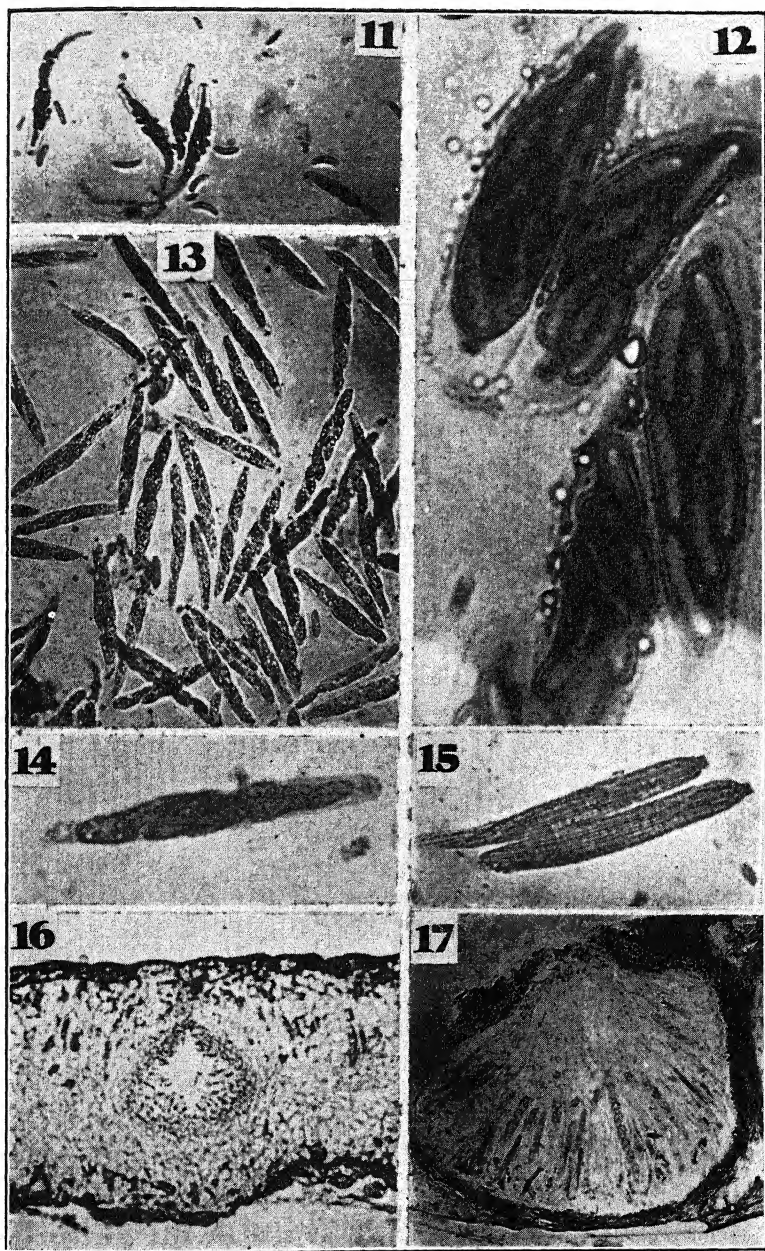
Figs. 11–17. Types of spores, asci and ascocarps in the Ascomycetes.

feldt's Ascohymeniales. This is also the old concept except for the Hysteriales in part, which are placed in the Pyrenomycetes in this revision. The Hypodermataceae are transferred to the order Helotiales as is done by Nannfeldt.

Inoperculates—*Ostropales and Helotiales*

These orders follow Nannfeldt's concept, and the difference between the ascus cap and threadlike canal in the former, and the wide pore of the latter can be easily distinguished. The characters of ascus and spores in the Ostropales are very similar to those of the Clavicipitaceae.

Operculates—*Pezizales and Tuberales*

The Pezizales here include the Pezizaceae and the Helvellaceae (Elvelaceae) as limited by Seaver (24). The Tuberales are placed at the end of the Discomycetes with the idea that they have evolved from the Pezizales. This is the usual position. It is very difficult to place them in a key with characters that will separate them from the hypogeic Plectomycetes.

PYRENOMYCETES

The writer includes in this subclass all fungi in which the asci are borne in parallel series in a closed fruiting body which ultimately opens by a definite pore or slit. The exception would be the Hypodermataceae in the Helotiales.

Sphaeriales

This order is revised to include only forms with the writer's (16) previous concept of a true perithecium; that is, walls arising from an archicarp, soon forming a globose hollow ball with a hymenium of asci and apically free paraphyses developing from a wall layer, and an ostiole formed by extension of the wall tissue and lined with periphyses. The mature asci have thin lateral and thick apical walls penetrated by a pore. The spores are typically liberated through the pore.

Young and mature perithecia are illustrated by Brown's figures (2: FIGS. 24–31) of *Xylaria,* and by the writer's figures 16, of

Phyllachora Lespedezae (Schw.) Sacc., 5, of *Cordyceps militaris* (L. ex Fr.) Lk., and 17, of *Phyllachora graminis* (Pers. ex Fr.) Fckl.

In making identifications, if there is more than one perithecium in the stroma, the presence or absence of a perithecial wall is sufficient. The difficulty usually comes in separating forms with single perithecia from uniloculate stromata, especially if one does not have access to early stages. The character of the ascus should separate these. If the ascus wall is apically thickened and possesses a pore, the fungus belongs in the Sphaeriales. If a pore is lacking and the outer wall splits when the spores are ejected, the fungus is a loculate form. An additional separation is that in the Sphaeriales the ascus gives a blue reaction in iodine, a character not present in the other orders.

CLAVICIPITACEAE

This family, with *Claviceps* Tul. as the type, constitutes a well defined group. There is the very marked cap at the apex of the long cylindric ascus (FIG. 8), and filiform spores that usually break up into segments at maturity. The pore is very narrow and penetrates the cap. The ascospores in ejection apparently do not pass through the pore, but instead the entire cap is pushed off and the spores extruded. The walls of the perithecia are pseudo-prosenchymatous, and are sharply defined in all species studied. The asci arise from a basal plectenchyma and never lie in a wall layer as in members of the other families. Paraphyses and periphyses are formed, but deliquesce early in most species.

Species of the genera *Claviceps, Cordyceps* (Fr.) Lk., *Balansia* Speg., *Dothichloë* Atk., *Ascopolyporus* Moll., and *Epichloë* Tul. have been studied, and in all cases the perithecia lie in a stroma which is either stalked or sessile. All are parasites on grasses or on insects.

Nannfeldt (l.c.) makes this group an order, and separates it from the rest of his Ascohymeniales by the lack of a special perithecial wall and paraphyses. This is at variance with findings of the writer. Figures 6–7, *Claviceps purpurea* (Fr.) Tul. and figure 5, *Cordyceps militaris* (L. ex Fr.) Lk. all show distinct walls.

In addition paraphyses are found in young perithecia. Jenkins (12) with *Cordyceps capitata* (Holmsk. ex Fr.) Lk. (*Cordyceps agariciformia* (Bolt.) Seaver) finds a true wall and ostiole and paraphyses-like threads and periphyses.

Luttrell (15), in reviewing the literature of the Hypocreales, mentions five types of development. His fourth type consisting of poorly developed wall and no paraphyses, and including the *Cordyceps-Claviceps* group, is hardly correct as there are walls and paraphyses, or "paraphyses-like threads" as Jenkins calls them, at an early stage. His fifth type, consisting of asci formed in locules in a stroma with perithecial wall, ostiole and paraphyses lacking—*Epichloë typhina* (Pers. ex Fr.) Tul., is also incorrect according to the writer's sections.

XYLARIACEAE

This is one of the oldest and best recognized groups of Ascomycetes. It consists of many genera and species widely scattered throughout the world. The entire series constitutes an excellent phylogenetic tree with few missing links. The members are peculiarly susceptible to changes in color, size, and shape of the stroma under variable environments, so the number of so-called species in the literature has been tremendous.

The perithecia have well defined pseudoprosenchymatous walls, and are either single or many in a stroma. The asci are cylindric, stalked, persistent, formed within a wall layer, possess a most peculiar crown at the base of the ascus pore, which ends in a wide plug (see FIG. 9, *Hypoxylon tinctor* (Berk.) Cke. and FIG. 10, *Xylaria Hypoxylon* (L. ex Fr.) Grev.). Spores are ejected through the pore into the perithecial neck and accumulate in black masses just outside of the ostiole. These spores are uniseriate, inequilaterally elliptical, 1-celled, dark colored, and have a longitudinal germ pore. Paraphyses are copious, filiform, branched, and persistent until late in the life of the perithecium, when they gelatinize, especially up near the ostiole.

Small 1-celled conidia, or probably in some cases spermatia, are borne on the surface of the stroma or in cavities.

The type of the *Xylariaceae* should be the genus *Xylaria* Hill. ex Grev. and the type species, *X. Hypoxylon*.

Nannfeldt (l.c.) designated this the most typical family for his Sphaeriales. It should be the type family as it exemplifies the concept most fully.

Genera that have been placed in other families by Lindau (6) such as *Anthostoma* Nits., *Anthostomella* Sacc., *Sordaria* Ces. & De Not., and *Rosellinia* De Not., have the special ascus crown character and so should go in the Xylariaceae.

The exceptional genus is *Clypeosphaeria* Fckl. In *C. mamillana* (Fr.) Lamb. all characters are well within the family concept, even to the possession of the ascus crown, but the spores are three-septate. Nannfeldt (l.c.) also noted the relationship to the Xylariaceae after studying *C. Notarisii* Fckl., the type.

Lindau (6) placed the genus *Xylobotryum* Pat., with two-celled ascospores, in this family. The writer has the type species, *X. andinum* Pat., and it has asci not thickened at the apex and neither crown nor pore, and has two-celled ascospores, which should exclude it.

ALLANTOSPHAERIACEAE

This family was created by von Höhnel (11) for all typical Pyrenomycetes with allantoid ascospores. He divided the family into four subgroups. One, the Diatrypeen v. Höhn., possesses asci located in a wall layer and which have a refractive ring. It belongs, according to Nannfeldt (l.c.), in the Sphaeriales. Further, he has included the genera *Cryptosphaeria* Grev., *Cryptovalsa* (Ces. & De Not.) Fckl., *Diatrype* Fr., *Diatrypella* (Ces. & De Not.) Sacc., *Eutypa* Tul., *Eutypella* (Nits.) Sacc., and *Quaternaria* Tul. Nannfeldt (l.c.) sets this group up as a family, the Diatrypaceae, and places it in the Sphaeriales. Wehmeyer (30) and also Wolf and Wolf (32) use the name Allantosphaeriaceae.

The writer thinks there will be less confusion if von Höhnel's name is retained and the group characterized as follows:

Perithecia pustulate or dispersed, usually sunken in bark or wood, with elongate necks, ostioles usually sulcate. Asci in a wall layer, clavate, with attenuate stalks, with thick apical walls, with refractive ring in pore, persistent; with ascospores chiefly 1-celled, allantoid, and light colored. Paraphyses filiform, branched, copi-

ous and persistent. Figure 11, *Eutypella fraxinicola* (Cke. & Pk.) Sacc., shows the characters of the ascus.

Conidia usually 1-celled, hyaline, borne on surface or in locules of stroma.

The type of the family should be *Diatrype* Fr., and of the genus *D. disciformis* Hoffm. ex Fr. Representative genera should be *Diatrype, Diatrypella, Eutypa,* and *Eutypella*. This is the concept of Wehmeyer (l.c.) for the family, except for the genus *Anthostoma,* which the writer places in the Xylariaceae because of the crown in the ascus.

DIAPORTHACEAE

This group was first delimited by von Höhnel (10) to comprise forms with his "diaportheen nucleus." Nannfeldt (l.c.) erected an order, the Diaporthales, coordinate with the Sphaeriales. Further, he placed the forms with allantoid spores but with the diaportheen centrum in another order, the Valsales. The writer retains essentially the von Höhnel grouping, or the more recent concept of Wehmeyer (30), without limiting it to forms on wood or bark.

The characters of the family consist of perithecia usually with long necks, one or more in a stroma or pseudostroma, or in no recognizable stroma, with rounded ostioles, not sulcate. Asci are in a wall layer, clavate, short-stalked, thin-walled except at the apex and that is very thick, penetrated by a pore containing a light-refractive ring, at maturity free from base and becoming loose in the perithecial centrum, completely filling it. In most cases spore ejection is through the pore, but sometimes the wall of the ascus dissolves and the spores collect in a gelatinous mass at the apex. Paraphyses are at an early stage seen as broad, free pointed, bands interspersed with some narrow filiform ones. The entire mass gelatinizes at maturity.

Conidia are usually borne in exposed layers, in acervuli, or locules in a stroma. They vary in shape and color as much as the ascospores.

The genus *Diaporthe* Nits. should be the family type and *D. eres* Nits. the type of the genus. The genera placed here include ones
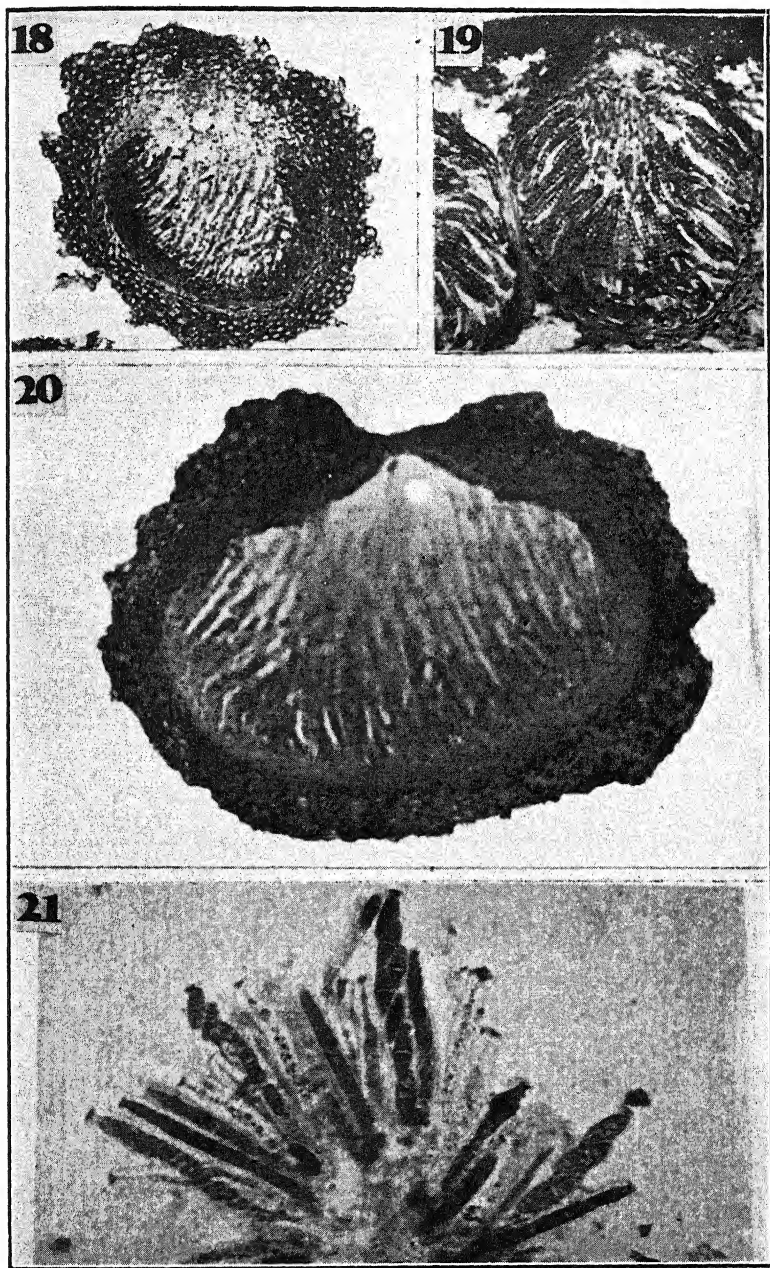
Figs. 18–21. Types of spores, asci and ascocarps in the Ascomycetes.

in both the Diaporthales and Valsales of Nannfeldt (l.c.), and in part ones in the families Clypeosphaeriaceae, Gnomoniaceae, Melogrammataceae, Melanconidaceae, and Valsaceae of Lindau (6). The chief genera are *Cryptospora* Tul., *Cryptosporella* Sacc., *Endothia* Fr., *Diaporthe* Nits., *Gnomonia* Ces. & De Not., *Gnomoniella* Sacc., *Hercospora* Tul., *Linospora* Fckl., *Mamiania* Ces. & De Not., *Massariovalsa* Sacc., *Melanconis* Tul., *Ophiodothella* v. Höhn., *Pseudovalsa* Ces. & De Not., *Valsa* Fr., and *Valsaria* Ces. & De Not.

Some of the above groups vary in at least one character. In *Linospora populina* Pers. ex Schrt., *Ophiodothella Ingae* (P. Henn.) Th. & Syd. and *Valsaria rubricosa* (Fr.) Sacc. the apical ring is very distinct, but the asci do not float out in water as easily as in *Diaporthe* species. Then in *Massariovalsa sudans* (Berk. & Curt.) Sacc. and *Pseudovalsa sigmoidea* (Cke. & Ell.) Sacc. the refractive ring is not plain.

The character of the asci floating out in water is shown in figure 13, *Diaporthe oncostoma* (Duby) Fckl., and the sessile ascus with the light refractive ring is illustrated in figure 14, *D. oncostoma*, figure 12, *Valsa ambiens* Pers. ex Fr., and in figure 15, *Cryptospora cinctula* (Cke. & Pk.) Sacc.

PHYLLACHORACEAE

This family has historically been placed in the Dothideales by Lindau (6), Stevens (25), and Theissen and Sydow (27). The type of the family is *Phyllachora* Nits., and of the genus *Ph. graminis* (Pers. ex Fr.) Fckl. The latter was supposed to have contained no true perithecia, but instead locules in a flat stroma sunken in the leaf (see Stevens [25], *fig.* 157). This concept was refuted by Orton (20), who showed that each perithecium contained a special wall, and that the blackened hyphae which form the clypeus arise in the epidermis or cuticle of the leaf as lateral proliferations of the ostiolar tissue.

There are a great number of specific names in the literature and all of them have been based on fungi found on living leaves of many plants. However, only the ones on grasses have been studied in detail and they conform to the type. Of the others, the

writer has studied only *Ph. Lespedezae* (Schw.) Sacc. (FIG. 16), and it has a true perithecium but in a stroma.

The characters of the family as delimited here follow:

Perithecia globose, with thin walls, lying in leaf tissue or in a stroma and maturing in living leaves, with plane ostioles and sometimes with a clypeus formed by the lateral proliferation of ostiolar hyphae. Asci persistent in a wall layer, cylindric, very short stalked, with walls fairly uniform in thickness, not especially thickened at apex, with wide pore in center of apex, blue with iodine. Ascospores uniseriate. Paraphyses copious, persistent, filiform, and branched.

This group contains at present only the genus *Phyllachora*. Whether other genera should be included depends on a thorough study of forms placed in the family by Theissen and Sydow (27) and others. Their separation of the old genus into several based on position of the fructification in the leaf does not seem to have merit.

Nannfeldt (l.c.) places *Phyllachora* in a family, the Polystigmataceae, which is based on *Polystigma rubrum* (Pers. ex Fr.) Chev. This forms a solid fleshy stroma with sunken perithecia in the leaf. The perithecia mature only after overwintering in the dead leaf. Whether this form should be placed in the same family with *Phyllachora* would depend on the results of more study.

Phyllachora species on grasses in the genera *Andropogen* L., *Elymus* L., *Panicum* L. and *Triodia* R. Br. have been studied by the writer, and they possess a true wall. At an early stage they show free paraphyses and asci growing into an inclosed hollow. Figure 17, *Phyllachora graminis,* shows these characters. Figure 16, *Ph. Lespedezae,* shows a very young perithecium in a solid fungous stroma, with typical characters of the Sphaeriales.

HYPOCREALES

This order is tentatively delimited here as set up by Lindau (6) with the exception of the Clavicipitaceae, which is placed in the Sphaeriales.

It has been thought that all of the forms were characterized by possession of a perithecial wall. For example, Clements and Shear
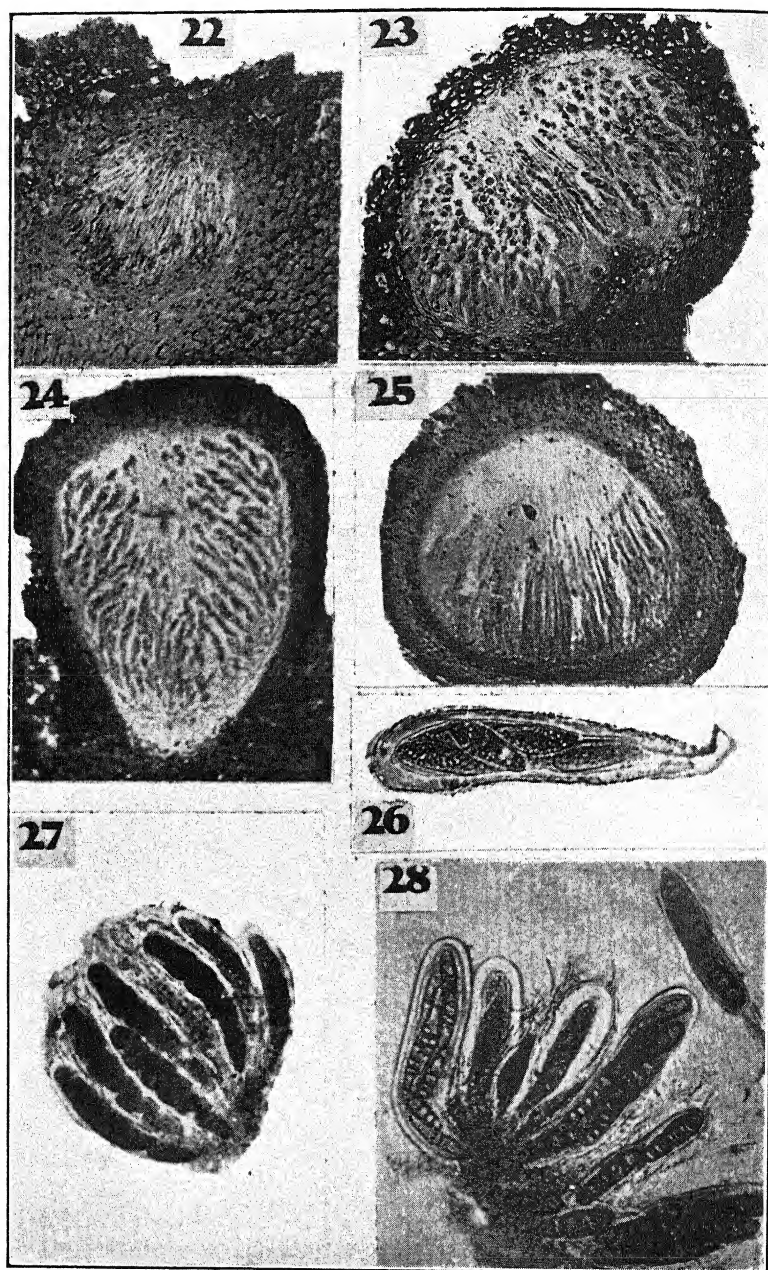
Figs. 22–28. Types of spores, asci and ascocarps in the Ascomycetes.

(4) say that the Hypocreales are distinguished from the Dothide-
ales by the presence of a distinct perithecial wall and fleshy bright
colored perithecia.   They make the group a family and place it in
the Sphaeriales.   The writer (18) has done the same thing in the
past.   However, in view of recent knowledge this may have been
a mistake, except for the Clavicipitaceae.   There are other large
groups such as species of *Nectria* Fr., *Hypomyces* (Fr.) Tul.,
Hypocrea Fr., and others whose life histories have not been studied
sufficiently to accurately judge their relationships.   It is possible
that there are other characters that would prevent this alignment
in the Sphaeriales.   For example, Luttrell (15) has made a very
detailed study of *Sphaerostilbe aurantiicola* (Berk. & Br.) Petch
and he finds that there is a true perithecial wall and a periphysate
ostiole, but no paraphyses, and there are vertical threads or pseudo-
paraphyses attached in the roof.   Here, then, are wall and ostiole
characters of the Sphaeriales combined with centrum characters of
the Pseudosphaeriales.   Luttrell also finds the vertical hyphae in
Nectria cinnabarina Tode ex Fr. and records similar conditions in
Nectria Ribis and *Hypocrea gelatinosa* (*Creopus gelatinosus* (Tode
ex Fr.) Lk.).   The writer finds this centrum in *Nectria cinna-
barina, Creopus gelatinosus, Nectria ochroleuca* (Schw.) Curt.,
Nectria coccinea Pers. ex Fr., *Nectria galligena* Bres., *Thyro-
nectria austro-americana* (Speg.) Seeler, *Hypocrea lenta* (Tode
ex Fr.) Berk. and Br. and *Hypomyces lactifluorum* (Schw. ex
Fr.) Tul.

The presence of the vertical threads has evidently been over-
looked in *Hypomyces, Hypocrea,* and *Podostroma* Sacc.   Both
Lindau (6) and Clements and Shear (4) state paraphyses lack-
ing for these genera.   In other cases these threads have been
called paraphyses.

Figures 18 and 20 show the vertical threads in *N. coccinea* and
figure 19 in *Hypocrea lenta.*   Figure 21 shows the pores in the
asci of *Nectria galligena.*

From the present insufficient evidence, then, it seems logical to
leave the Hypocreales, with the exception of the Clavicipitaceae,
in the status quo until more work has been done.   Then if most of
them do have the above characters we will have an order charac-

terized by the presence of a definite perithecial wall, but with the centrum of the Pseudosphaeriales. At present all forms with free paraphyses should be removed to the Sphaeriales, and those with asci in pseudoparenchyma to the Dothideales, and those with the vertical threads but no wall to the Pseudosphaeriales.

Dothideales

In revising this group no change is necessary in the original concept of the type. The name came originally from *Dothidea* Fr., and *Dothidea Sambuci* Fr. is the type of the genus. This species is not the first one described, but it is the element with which the name has been permanently associated, and it fixes the generic name, and also the family and ordinal names as commonly applied. Theissen and Sydow (27) did not have sufficient basis to change this name to *Systremma* Th. & Syd.

The present idea, then, is in reality the very old one of ascus locules in a stroma with no special wall, and each locule, as in *Dothidea Sambuci,* contains a persistent fascicle of asci with no interthecial threads. The locule opens by a lycigenously formed pore in the stromal wall. This concept is emended to include uniloculate forms with this centrum. The ascus is uniformly thickened and opens as in the Pseudosphaeriales by the outer wall splitting and the inner protuding. (See Wolf and Wolf **32**: *fig.* 80.)

Theissen and Sydow (**29**) place the uniloculate stromata such as in *Mycosphaerella* Johans. in the Pseudosphaeriales, but as pointed out previously the interthecial tissue is ontogenetically different in the two concepts.

In the group as monographed by Theissen and Sydow (27) the Phyllachoraceae go in the Sphaeriales and all forms with pseudoparaphyses, such as *Auerswaldiella puccinioides* (Speg.) Th. & Syd., must be transferred to the Pseudosphaeriales as emended by the writer.

At present one could arbitrarily separate the group into two families as follows: multiloculate stromata, Dothideaceae; uniloculate stromata, Mycosphaerellaceae. This will not always maintain a sharp separation because there are species of *Mycosphaerella*

such as *M. effigurata* (Schw.) House in which the stroma often contains more than one locule. This is also true of *Guignardia Bidwellii* (Ell.) Viala & Ravaz.

The multiloculate stroma is shown in figure 33, *Dothidea collecta* (Schw.) Ell. & Ev., and figure 32, *Auerswaldia examinans* (Mont. & Berk.) Sacc. Figure 34, *Dothidea collecta,* shows the ascus walls with no pore and the basal attachment producing the fascicle. Figures 35–36, *Mycosphaerella effigurata,* show the characteristic centrum and ascus.

PSEUDOSPHAERIALES

This order is based in part on the Pseudosphaeriaceae concept of von Höhnel (9), Theissen and Sydow (29), Petrak (21) and others. The writer (16) discussed this question in detail some time ago, and there is the more recent discussion by Nannfeldt (19).

Previously the writer has thought that the threads (see FIG. 22) in the centrum attached at top and bottom were remnants of stromal tissue in process of dissolution by developing asci. Since then sections of young stages of many species have shown the presence of these threads before the asci even begin to form. The archicarp first develops in the stroma, followed by a fan-like downward growth of threads from the position of the archicarp. Then as the stroma develops the threads elongate and asci arise at their bases. The connection between these vertical threads and the asci has never been satisfactorily explained. Sartoris and Kauffman (23) and Cavara and Mollica (3) thought the young asci were formed in the threads. Dodge (5) with a *Leptosphaeria* and Wehmeyer (31) with a *Pseudotrichia* thought there was no connection with the asci.

This stage in the ontogeny has not been found in the group delimited here as the Sphaeriales. Other characters that set the Pseudosphaeriales apart are the thick ascus wall not penetrated by a pore, and the method of splitting of the outer wall for spore ejection.

This order, then, is characterized by the asci being borne in locules, in a wall layer or between pseudoparaphyses, and by one
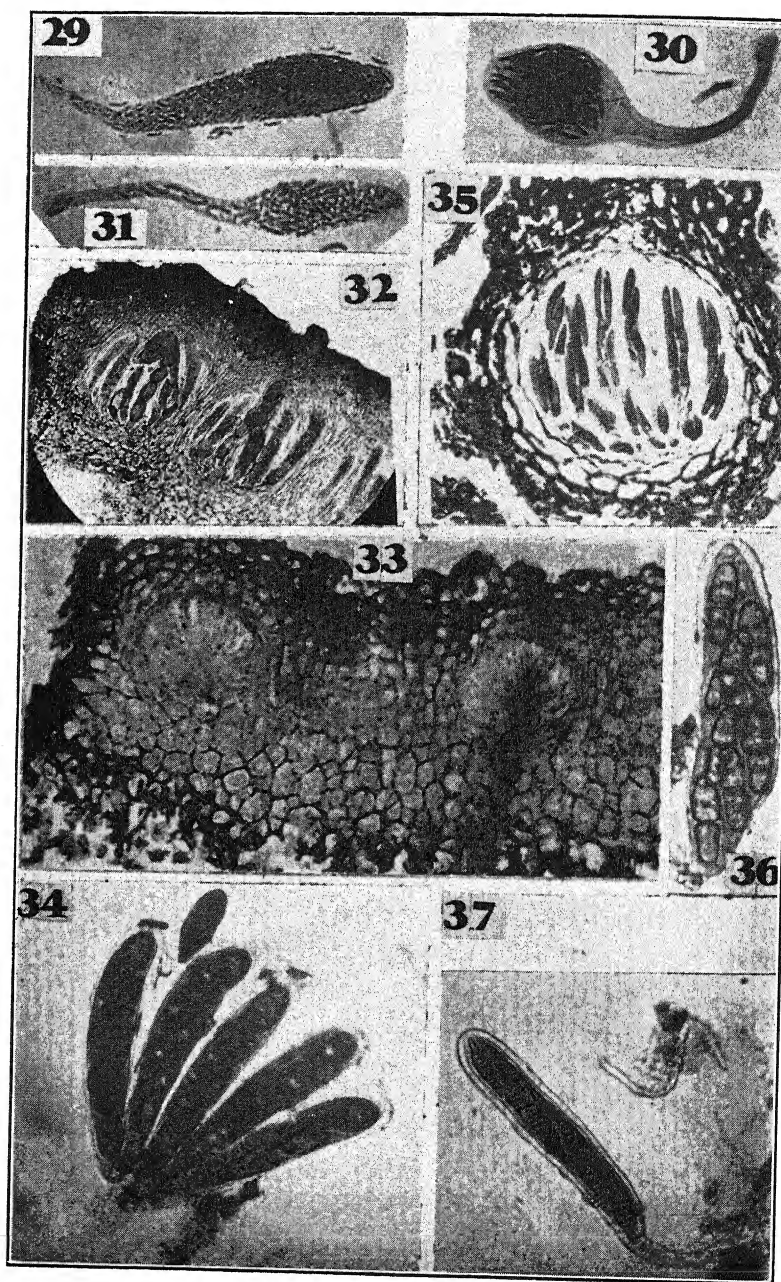
Figs. 29–37. Types of spores, asci and ascocarps in the Ascomycetes.

to many locules in a stroma opening by a lysigenously formed pore. The thickness of the locule wall is no criterion of the stromal character. The wall cells tend to be pseudoparenchymatous. The other characters are the connected threads and the uniformly thickened ascus wall with no pore.

Genera placed here are ones previously found chiefly in Lindau's (6) families, Sphaeriaeae, Amphisphaeriaceae, Pleosporaceae, and Cucurbitariaceae, and in the Dothideales of Theissen and Sydow (27). Species studied by the writer and found to have this centrum have been enumerated in the Plant Disease Reporter (18).

At present there seem to be two tendencies that might well represent families:

Ascus walls thick, pseudoparaphyses not gelatinizing at maturity
          Pleosporaceae

Ascus walls uniformly thin, pseudoparaphyses gelatinizing at maturity, forming a jelly-like mass in the roof of the stroma
          Nitschkiaceae

Mature locules are shown in figure 23, *Auerswaldiella puccinioides,* figure 24, *Dibotryon morbosum* (Schw. ex Fr.) Th. & Syd. and figure 25, *Lasiosphaeria ovina* (Pers. ex Fr.) Ces. & De Not. All of them show the attached threads. The ascus characters of thick wall and no pore are shown in figure 26, *Botryosphaeria Ribis* (Tode ex Fr.) Gr. & Dug., figure 27, *Pleospora aurea* Ell., and figure 28, *Massaria Argus* (Berk. & Br.) Fres.

The forms placed in the Nitschkiaceae, figure 29, *Fracchiaea heterogena* Sacc., figure 30, *Coronophora ootheca* (Berk. & Curt.) Sacc., and figure 31, *Cryptosphaerella annexa* (Nits.) v. Höhn., have allantoid spores and the asci show no pore. The latter constitutes an easily recognizable character distinguishing them from the Allantosphaeriaceae or Diaporthaceae.

MICROTHYRIALES

The writer prefers this name to that of Hemisphaeriales because the Microthyriaceae with the shield-shaped ascocarp of radiating structure should be the type group. Only a few of the species have been investigated morphologically; however Nannfeldt (l.c.) placed

them in his Ascoloculares with the monascus locule.   Luttrell (14) with *Myiocopron Smilacis* (De Not.) Sacc. found the ascocarp to be an ascostroma containing a single locule filled with asci and pseudoparaphyses.   Then in *Morenoella quercina* (Ell. & Mart.) Th., Luttrell (13) found that the ascocarp is also a stroma and that each ascus originates individually and by its expansion creates a locule within the stroma.   He suggests that possibly all members of the order described as "aparaphysate" are of this type.

The order Microthyriales, then, should be limited to species with dimitate stromata bearing locules, one or more, with asci between pseudoparaphyses.   In this manner they would be closely related to the Pseudosphaeriales.

Forms now in this order with shield shaped ascocarps, in which the asci form single locules by dissolving stromal elements, and which have no pseudoparaphyses, should be connected with the higher Myriangiales such as the Dothioraceae.

Lophiostomatales

The writer has made no detailed study of any members of this order, but places them in a separate order rather than as a family in the Sphaeriales (see Lindau, 6), because of the presence of pseudoparaphyses and asci with no pore.   This order then should comprise uniloculate perithecial-like stromata having connected pseudoparaphyses and opening by a slitlike ostiole.   These fungi differ from the Pseudosphaeriales only in the opening.

Nannfeldt (l.c.) has these in his Ascoloculare group because of the threads in the centrum.

Hysteriales

The type of the order is the family Hysteriaceae obtained from *Hysterium* Tode ex Fr., which has *H. pulicare* Pers. ex Fr. for the generic type.   The species here have boat-shaped ascocarps, opening by an elongate slit.   The asci (FIG. 37, *Hysterographium flexuosum* (Schw. ex Fr.) Rehm) are of the Pseudosphaeriales type with uniformly thickened wall and no pore, and lie between pseudoparaphyses.

Nannfeldt (l.c.) placed the family Hysteriaceae in his Pseudo-sphaeriales order in the Ascoloculares. This is after he had taken out the Hypodermataceae and transferred them to the Asco-hymeniales.

The Hysteriales could be derived from the Pseudosphaeriales through the Lophiostomatales. There are forms, such as those of *Bulliardella* Sacc., which are intermediate in ascocarp characters.

<div align="center">TENTATIVE KEY TO THE ASCOMYCETES</div>

1. Asci not in a specialized fruiting body, but borne singly or clustered on the mycelium . . . . HEMIASCOMYCETES
   2. Mycelium usually poorly developed, growth by budding common.
      3. Saprophytic; asci formed directly by fusion of cells. . *Endomycetales*
      3. Parasitic on plants; usually causing hypertrophy; asci arising from chlamydospores . . . . *Exoascales*
1. Asci borne in ascocarps . . . . EUASCOMYCETES
   4. Ascocarps closed.
      5. Opening only by weathering; *asci globose* to *broadly clavate*, with walls uniformly thickened, with *no pores*, borne in tufts or irregularly arranged . . . . PLECTOMYCETES
         6. Asci borne singly in locules composed of ascogenous hyphae when they are at irregular levels.
            7. Stroma lacking . . . . *Eurotiales*
            7. Stroma present . . . . *Myriangiales*
         6. Asci arising singly or in tufts from a basal plectenchyma
                                            Erysiphales
      5. Ascocarps opening by a definite small pore or slit; *asci clavate* to *cylindric* . . . . PYRENOMYCETES
         8. Perithecia stalked, borne on a receptacle; neither paraphyses nor periphyses; minute parasites on insects. *Laboulbeniales*
         8. Perithecia not stalked.
            9. Asci with *apical pore*, in *perithecia*; perithecia one to many in a stroma, with a differentiated wall, with ostiole formed by the extension of the wall and lined with periphyses
               10. Asci within a wall layer of *apically free* paraphyses, thin-walled except for apex . . . . *Sphaeriales*
               10. Asci within a wall layer of *vertical hyphae* (pseudo-paraphyses) connected in roof of perithecium, with wall uniformly thickened . . . . . . *Hypocreales*
            9. Asci in a *uniloculate* or *multiloculate stroma*, opening by an apical lysigenously formed pore; with no periphy-ses and no differentiated wall to the ascal centrum. Ascal wall uniformly thickened, with *no pore, opening by the outer wall splitting and the inner protruding.*

   11. Ascocarp opening by a circular pore.

      . 12. Asci in basal fascicle in the stroma with no interthecial threads............*Dothideales*

      12. Asci with pseudoparaphyses connected at top and bottom of locule.

         13. Hymenium in wall-layer, ascocarp variously shaped, not dimitate

                       Pseudosphaeriales

         13. Hymenium in flat, basal layer, ascocarp dimitate...............*Microthyriales*

   11. Ascocarp opening by an elongate slit, pseudoparaphyses in locule.

         14. Ascocarp perithecial-like.....*Lophiostomatales*

         14. Ascocarp boat-shaped............*Hysteriales*

4. Ascocarp open.   Hymenium either exposed from the first, or inclosed and later becoming more or less widely exposed, or if opening only by weathering then hypogeic; paraphyses apically free; ascocarps variously shaped apothecia......................Discomycetes

   15. Asci inoperculate, without a definite method of opening or opening by a pore.

      16. Asci cylindrical, apically obtuse with thickened membrane penetrated by a canal, with filiform spores; spores septate, breaking into cylindrical parts................*Ostropales*

      16. Asci and ascospores not as above..............*Helotiales*

   15. Asci operculate, opening by a lid.

      17. Ascocarp epigeic at least at maturity, hymenium exposed from the first or inclosed and later opening.....*Pezizales*

      17. Ascocarp hypogeic, hymenial layer exposed by weathering

                          Tuberales

A KEY TO THE SPHAERIALES

1. Ascus with thickened apical cap, penetrated by a narrow, threadlike pore; spores filiform and breaking into segments.............*Clavicipitaceae*
1. Ascus without cap, pore not as above, spores of various shapes, if filiform, not breaking into segments.

   2. Asci very thick at apex, pore well defined.

      3. Inner wall of ascus producing a crown at base of pore, with latter ending in a wide plug.   Asci long stalked, persistent; paraphyses persistent, filiform and branched; ascospores 1-celled, dark, germinating by a longitudinal germ pore, uniseriate.*Xylariaceae*

      3. Crown not present, a light-refractive ring in pore.

         4. Asci long stalked, persistent, paraphyses persistent, filiform and branched; ascospores chiefly 1-celled, allantoid; ostiola sulcate............................*Allantosphaeriaceae*

         4. Asci short-stalked, not persistent, soon floating loose in cavity of perithecium, spores of various shapes, paraphyses as broad bands, gelatinizing at maturity; ostiola not sulcate

                         Diaporthaceae

2. Asci not especially thickened at apex, wall of uniform diameter, with pore not very evident.

5. Fungi in living leaves, with a stroma, or only forming a darkened clypeus by proliferation of ostiolar tissue; perithecia within leaf, with pseudoprosenchymatous walls; ascospores uniseriate; paraphyses copious and persistent..............*Phyllachoraceae*

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EXPLANATION OF FIGURES

Fig. 1. Asci of *Taphrina caerulescens.* Fig. 2. *Myriangium Duriaei,* single globose ascus. Fig. 3. *Dothiora subtropica,* asci in stroma, representing high point in Myriangiales with asci in single layer and tending toward the cylindric. Fig. 4. *Microsphaera Alni,* semi-globose ascus. Fig. 5. Longitudinal section of perithecia of *Cordyceps militaris,* showing definite wall. Fig. 6. Cross section of fertile stroma of *Claviceps purpurea,* also showing walls to perithecia. Fig. 7. Longitudinal section of *Claviceps purpurea.* Fig. 8. Ascus of *Cordyceps capitata,* showing cap and narrow pore. Figs. 9, 10. Asci of *Hypoxylon tinctor* and *Xylaria Hypoxylon,* showing crown and wide pore in apices. Fig. 11. Asci of *Eutypella fraxinicola,* showing stalks and refractive ring in pores. Figs. 12, 13, 14 and 15. Asci of *Valsa ambiens, Diaporthe oncostoma* (13, 14) and *Cryptospora cintula,* all showing the lack of stalk, and the refractive ring in the pore. Fig. 16. Young perithecium of *Phyllachora Lespedezae,* showing definite wall,

free paraphyses and asci growing into a cavity. FIG. 17. Perithecium of *Phyllachora graminis,* showing the wall with no stroma, and asci and free paraphyses. FIG. 18. Young perithecium of *Nectria coccinea,* showing vertical threads attached at top and bottom and a wall layer of very young asci. FIG. 19. Mature perithecium of *Hypocrea lenta,* showing the remnants of the vertical threads just before gelatinization. FIG. 20. A medium aged perithecium of *Nectria coccinea,* showing attached vertical threads and young asci. FIG. 21. *Nectria galligena,* showing asci with pores and wide plugs. FIGS. 22–23. *Auerswaldiella puccinioides.* Immature locule of figure 22 shows vertical threads and asci in or at their bases, while figure 23 is a mature locule with the attached threads still showing. FIG. 24. Single ascus locule of *Dibotryon morbosum,* showing attached threads between the asci. FIG. 25. *Lasiosphaeria ovina,* also showing attached threads. FIG. 26. An ascus of *Botryosphaeria Ribis,* with thick wall and no apical pore. FIG. 27. An ascus centrum of *Pleospora aurea,* showing asci with thick walls and no pores and the vertical threads. FIG. 28. Asci of *Massaria Argus,* also showing thick walls and no pores. FIG. 29. *Fracchiaea heterogena.* FIG. 30. *Coronophora ootheca.* FIG. 31. *Cryptosphaerella annexa.* All three asci have allantoid spores, but the walls are not thickened at the apices and there is no pore. FIG. 32. A multiloculate stroma of *Auerswaldia examinans* with asci growing up in the stroma with no paraphyses nor vertical threads. FIG. 33. *Dothidea collecta,* showing the same locule centrum characters as figure 32. FIG. 34. Asci of *Dothidea collecta,* showing uniformly thickened walls with no pores, and the basal attachment producing the fascicle characteristic of the order. FIG. 35. *Mycosphaerella effigurata,* showing the same type of locule centrum as in the *Dothidea* species. FIG. 36. Ascus of this *Mycosphaerella,* also showing characters typical of those of the *Dothidea.* FIG. 37. Ascus of *Hysterographium flexuosum,* showing the uniformly thickened wall and no pore.

THE "DUAL PHENOMENON" AND TRI-CHOPHYTON MENTAGROPHYTES [1]

WILLIAM J. ROBBINS AND ILDA MCVEIGH

(WITH 1 FIGURE)

Wilhelm (3) has applied the concept of "dual phenomenon" developed by Snyder and Hansen to the dermatophytes. He assumes that a species of a dermatophyte is composed of races and each race is dual, that is, composed of two distinct constituents associated together in culture. One constituent produces conidia in abundance but relatively scanty mycelium; this he designates as the C or conidial constituent. The other produces fewer conidia but more abundant mycelium; this he designates as the M or mycelial constituent. The M type, he says, arises as a mutation in old cultures of the C type even though the culture is started from a single conidium.

In addition to the C and M constituents, a "race" of a dermatophyte may give rise to forms culturally intermediate between C and M. Wilhelm suggests that in old cultures the mycelium and spores become heterocaryotic with respect to C and M nuclei. The variation exhibited upon single spore analysis of these old cultures results from chance separation of nuclear types in the spores at the time they are formed. He concludes that the normal and pleomorphic forms of *Trichophyton mentagrophytes* pictured by Robbins and McVeigh (2) may be considered respectively as C and M types.

We interpret Wilhelm's suggestion to mean that given a race of a dermatophyte in the conidial form, the following occurs: The conidial type, C, mutates and forms the mycelial type, M. Heterocaryosis occurs between the C and M, that is, mycelium develops

containing both C and M nuclei; this form is intermediate in character between the C type and the M type. If single spore isolations are made from the C type, only C is obtained because only C nuclei were present. Similarly, only M is obtained from M. However, single spore isolations from intermediate forms, CM, yield the conidial type, the mycelial type, and, in addition, intermediates if the spores isolated are multinucleate.

Our experience with the variants which occur in older cultures of *T. mentagrophytes* has led us to doubt that the concept of a "dual phenomenon" supplemented with heterocaryosis and the separation of nuclei is an adequate explanation for the occurrence and characteristics of the variants which can be isolated from old cultures of this fungus. In the first place, the number of variants which can be isolated from a single "race" appears to us to be greater than can be accounted for by heterocaryosis. In the second place, we have found that the variants can be maintained indefinitely in culture provided transfers are made to fresh media at relatively short intervals—a week or thereabouts. If any of the variants isolated from old cultures of *T. mentagrophytes* were heterocaryotic, we would scarcely anticipate that they could be maintained in culture without evidencing nuclear separation since many of them form spores freely before subcultures are made.

The application of the concept of "dual phenomenon" to *T. mentagrophytes* was tested experimentally by isolating nine variants from an original conidial culture which had been obtained from a human subject. Colonies of the nine variants and the original form grown on peptone agar and asparagine agar are shown in figure 1. They ranged from a highly mycelial rapidly-growing form which produced conidia slowly to highly conidial slow-growing forms. Since these variants were obtained from old cultures of the conidial form on which pleomorphic colonies had developed, it would seem justifiable to assume that some of the isolations were intermediates, as defined by Wilhelm.

Each of the ten isolations was maintained at approximately 35° C. on peptone agar by making transfers to fresh media at short intervals. Single spore isolations from each were made by spreading spores thinly on the surface of an agar plate. After one or

two days incubation, individual microconidia which had begun to germinate were selected (using a binocular or the lower power of a compound microscope), and transferred to tubes.   Comparisons were made of the resulting colonies.

A description of the origin of each of the isolations and their general characteristics, together with the results obtained on isolations of single microconidia, is given below.

Several media were used as follows. Asparagine-peptone agar contained per liter 50.0 g. dextrose, 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7H_2O$, 1500 mμ moles thiamine, 2.0 g. asparagine, 1.0 g. neopeptone, 0.5 ml. mixture of mineral supplements (1), and 15.0 g. agar.   Peptone agar was prepared as above except that it contained 2.0 g. per liter of neopeptone and no asparagine.   Asparagine agar was the same as the asparagine-peptone agar with peptone omitted.   Ammonium nitrate agar was identical with the asparagine-peptone agar except 2.0 g. of NH_4NO_3 was substituted for the asparagine and peptone.   The pH of these media ranged between 5.0 and 5.6.

The morphology of the colony, pigment production, conidial formation and microscopic character of a particular isolate are affected by the composition of the medium and by the temperature and time of incubation.   In our experience, the distinctions between isolates, at least so far as colony appearance is concerned, are sharper if the medium is not too rich.   We have, therefore, made our comparisons on media containing relatively small amounts of nitrogen.

The (N) form

T. mentagrophytes was originally isolated from a human subject in August, 1942.   It was a freely sporulating form with sparse mycelial growth corresponding to the condial type described by Wilhelm.   We have referred to this as the normal (N) strain.   A single spore culture was made and maintained in a freely sporulating condition up to April 24, 1947, on asparagine-peptone agar by subculturing to fresh media at intervals which did not exceed two weeks.   Since the date given, it has been maintained on peptone agar by transferring at weekly intervals to fresh media.

Microscopic examinations of cultures of (N) grown on peptone agar at 35° C. made at the end of one and two weeks, showed the mycelium to be variable; both slender and wide hyphae occurred. Microconidia were numerous in one week. Chains of chlamydospores were common. No macroconidia were observed in two weeks on this medium.

The (N) form grows slowly on an asparagine medium, more rapidly on peptone. Little or no growth occurs with NH_4NO_3 as source of nitrogen.

When single spore isolations were made from the (N) form, they developed into (N) cultures.

In February, 1946, sixty single spore isolations from a young culture of (N) were made to asparagine-peptone medium. Subcultures of each isolation were made to NH_4NO_3 agar. The isolations on asparagine-peptone agar were indistinguishable when examined after seven and nine days, and those on NH_4NO_3 gave characteristic response for (N) on that medium.

On May 3, 1948, sixty-nine single spore isolations were made to peptone agar from a three-day old (N) culture which had grown on peptone agar at 35° C. All proved to be identical in appearance when examined after ten days. Subcultures of each isolation were made after four days to an asparagine medium. All appeared to be alike after six days growth.

If cultures of the (N) strain are allowed to age, variants develop which can be distinguished by rate of growth, morphology of colony, pigment production, microscopic appearance and physiological characteristics. Some of these have been described earlier (1, 2). Although no particular effort has been made to discover how many variants could be isolated, it is our impression that they would be considerable in number—fifty or more. In any event, those which we have isolated have been maintained in culture apparently unchanged over considerable periods by transferring to fresh media at intervals of a week or thereabouts.

The (B) variant

A strongly mycelial type was isolated by mass transfer from a pleomorphic colony which had developed on an (N) culture. A

single spore isolation was made and labeled (B). It has been maintained in culture for over three years.

In February, 1946, forty-six single spore isolations of (B) were made to asparagine-peptone agar. Subcultures of each of the isolations were made to NH_4NO_3 agar. All isolations on peptone agar appeared to be alike, and the subcultures to NH_4NO_3 agar were indistinguishable one from another.

On May 5, 1948, sixty-five single spore isolations were made from a thirteen-day old culture of (B) to a peptone medium, and subcultures were made from each isolation after six days to an asparagine medium. Examinations made up to twelve days on the peptone and six days on the asparagine medium showed all isolations to be indistinguishable.

The (B) form is the most rapidly growing form we have isolated. On peptone and asparagine agar it produces a thick, white, fluffy colony. It is able to use NH_4NO_3 though growth is less rapid than with asparagine or peptone.

Microscopic examination of cultures grown at 35° C. on the peptone medium were made at the end of one, two, three and four weeks. At the end of one week, the hyphae were slender and uniform in width. No micro- or macroconidia and no chlamydospores were observed. A few hyphae had swollen tips. On the older cultures, some thicker hyphae were noted and some of the slender hyphae developed thickened portions, but no chains of chlamydospores were found. A few microconidia had developed by the end of two weeks and the number increased until they were numerous after four weeks. A few macroconidia were observed in cultures four weeks old.

The (A) variant

A mycelial type was isolated from a pleomorphic colony on a plate inoculated with (N). A single spore isolation was made and the resulting culture labeled (A). It was less vigorous than (B). It produced conidia more freely than (B) but much less so than (N). The hyphae were variable in diameter but the proportion of wide hyphae was less than in cultures of (N). Macroconidia were uncommon up to four weeks and no chains of chlamy-
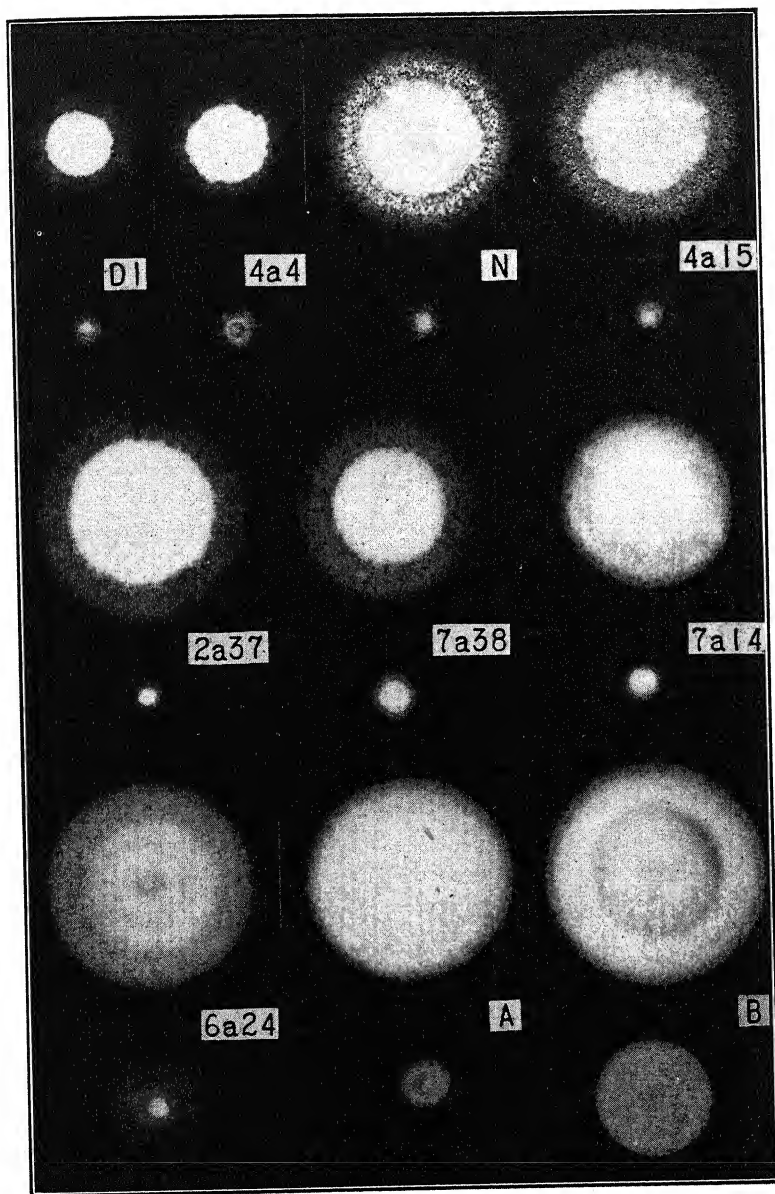
FIG. 1. A conidial race (N) of *Trichophyton mentagrophytes* and nine variants derived from it grown at 35° C. for twelve days on peptone agar and eleven days on asparagine agar. For each strain the growth on asparagine agar is below; on peptone agar, above.

dospores were observed though occasional swellings in the hyphae occurred.

In January, 1948, 218 single spore isolations of (A) were made to asparagine agar from seven-, eight- or nine-day-old cultures on peptone agar. After seven or eight days, transfers were made to peptone agar. All cultures appeared to be alike. Transfers were made from the peptone cultures to an asparagine medium. One week later all cultures looked alike. None resembled (N) or (B).

On April 29, 1948, fifty-six single spore isolations were made to peptone agar from an (A) culture which had grown seven days at 35° C. on peptone agar. All these isolations appeared alike up to fourteen days. Transfers were made to asparagine agar from each isolation after six days on peptone agar. When examined six days later, all cultures looked alike.

Variant (6a24)

A mass isolation was made from a pleomorphic area appearing on an agar plate nine days after inoculation with a spore suspension from a seven-day-old culture of (N). This was labeled (6a) and thirty-six single spore isolations were made on peptone agar. All of these appeared alike after eleven days. One of these isolations, (6a24), was selected for further study. On May 10, 1948, sixty-five single spore isolations of (6a24) were made from a four-day-old culture to peptone agar and after four days, subcultures of each were made to an asparagine agar. Observations made after eleven days growth on the peptone agar indicated that all of these cultures were alike in gross morphology. The subcultures on the asparagine medium appeared alike after one week.

Microscopic examination showed that this isolation resembled form (B) in being composed of slender hyphae. However, in the production of conidia (6a24) resembled the (N) form. Microconidia were very numerous in a culture grown on peptone agar for one week. Chains of chlamydospores were uncommon.

Macroscopically, (6a24) on peptone agar was much like the (A) and the (B) form. It consisted of a rapidly spreading, thick, aerial mycelium. Its growth characteristics on an asparagine medium, however, were different from those of any of the forms studied.

On this medium, it formed a small central area of heavy mycelium with a wide area of sparse subsurface mycelium.

Variant (7a38)

A mass isolation from a pleomorphic colony appearing on a plate originally inoculated with a seven-day-old culture of (N) was labeled (7a). The cultures which developed from thirty-two single spore isolations made on peptone agar were of two types. Both types were faster growing and less powdery than the (N) form. They resembled the (A) and (B) forms in being fluffy. One type produced considerable yellow pigment in the peptone agar and formed a small powdery area at the upper area of the slant. Twenty-one of the isolations were of this type, and a single one, (7a38), was selected for further study. The remaining eleven isolations were fluffy over the entire surface and developed little or no pigment. This type spread less rapidly than the first described. One of this form, (7a14), was reserved for further study.

On May 6, 1948, seventy single spore isolations were made from a six-day-old culture of (7a38) to a peptone medium. Minor differences in pigmentation and colony size were noted when the cultures were first observed. Observations after eleven days showed all to be of one type. Subcultures of each of the isolations were made to asparagine agar five days after isolation. On the asparagine medium, all were alike after six days.

Microscopically, (7a38) resembled the (N) form. The mycelium was composed of both slender and wide hyphae. Both microconidia and chains of chlamydospores were present in a week-old culture. The conidia were numerous.

(7a38) on plates of peptone agar was intermediate between the (N) and (B) forms in appearance. It formed a heavier growth than (N) but less than (7a14). On the peptone agar, the most distinguishing characteristic was the production of yellow pigment. It grew slightly more rapidly on the asparagine agar than (7a14).

Variant (7a14)

On May 10, 1948, seventy single spore isolations were made from a five-day-old culture of (7a14) to peptone agar. After ten

days, all seventy of the isolations appeared alike. Six days after isolation, subcultures of each were made on asparagine agar. Observations made six days later indicated that all were alike in gross morphology.

The mycelium of (7a14) was composed of very slender hyphae. Microconidia in a week-old culture were more numerous than in the (A) form. There were occasional swollen hyphae similar to those observed in the (A) form. No chains of chlamydospores were observed.

On peptone agar plate cultures, (7a14) in general appearance was much like the (B) form except that it did not spread so rapidly. On asparagine plates, (7a14) grew less rapidly than (B). It formed a small area of dense growth with thin subsurface radiations.

Variant (2a37)

A mass isolation from a pleomorphic colony on a plate originally seeded with (N) was labeled (2a). Fifty-five single spore isolations from this culture made on peptone agar appeared to be alike after nine days growth. One of these, (2a37), was selected for further study. Sixty-eight single spore isolations were made to peptone agar from a five-day-old culture of (2a37) on peptone agar. All appeared to be alike after thirteen days growth. Transfers of each isolation were made to asparagine agar after six days on the peptone agar. All appeared to be alike after six days growth on the asparagine agar. (2a37) was less vigorous on peptone agar than (B), (A), (6a24), (7a14) or (7a38). Growth was heavier and less powdery than (N).

Microscopic examinations of (2a37) were made after one and two weeks growth at 35° C. on peptone agar. The hyphae were variable in width; the thicker hyphae predominated at the end of two weeks. Microconidia were numerous at the end of one week. Chains of chlamydospores were rare at the end of one week and more numerous but not common after two weeks. Macroconidia were infrequent.

Variant (4a15)

A mass isolation made from a pleomorphic colony which had developed on a plate originally seeded with (N) was labeled (4a). Thirty-two single spore isolations were made from this culture to peptone agar. Two types of colonies were distinguishable, both powdery and in general resembling (N). One type grew somewhat more rapidly on peptone agar than (N); the other less rapidly. A single isolation, (4a15), of the first group, and one, (4a4), of the second group, were selected for further study.

On May 10, 1948, sixty-one single spore isolations were made to peptone agar from a culture of (4a15) which had grown for five days at 35° C. on peptone agar. After fourteen days, all isolations appeared to be alike. Transfers were made to asparagine agar from each isolation at the end of seven days. After seven days, all cultures on asparagine agar appeared alike.

Macroscopically and microscopically, (4a15) resembled (N) closely. It is distinguishable on asparagine agar where the growth is less powdery and heavier than (N).

Variant (4a4)

On May 7, 1948, sixty-eight single spore isolations were made to peptone agar from a culture of (4a4) which had grown for six days at 35° C. on peptone agar. On examination twelve days later, sixty-five isolations appeared to be alike and three seemed somewhat more fluffy. Subcultures of these three and three of the others were made to peptone agar and proved to be indistinguishable. After six days, transfers of each isolation were made to asparagine agar. Six days later, all cultures on asparagine agar appeared to be alike.

Form (4a4) was less vigorous on peptone agar than (N) but somewhat more so than (D1). Microscopic examinations were made of cultures grown on peptone agar at 35° C. after one and two weeks. The hyphae were variable but wide ones predominated. Microconidia and chains of chlamydospores were numerous. Macroconidia were not observed.

Variant (D1)

A strongly conidial type, slower growing than (N), was isolated from plates of asparagine-peptone agar which had been inoculated from a seven-day culture of (N) and incubated for fifty-four days at 35° C. Evident pleomorphisms had developed on this plate. A spore suspension was made and plated on asparagine-peptone agar. Three types of colonies developed—namely, pleomorphic forms, (N) forms, and some slower growing than (N). An isolation was made May 1, 1946, from one of the slow-growing colonies and labeled (D). A single spore isolation was made from (D) and labeled (D1). This variant has been maintained in culture to date by making transfers to fresh media at seven-day intervals. On May 5, 1948, seventy-eight single spore isolations were made to peptone from a five-day-old (D1) culture growing on peptone agar. All seventy-eight isolations proved to be identical in gross morphological appearance when examined after twelve days. After six days on peptone, transfers from each of the seventy-eight isolations were made to asparagine agar. All were identical in appearance when examined after six days incubation. When first examined, six of the subcultures on asparagine agar appeared slightly more fluffy than the balance. Transfers of these six and some of the other seventy-two were made to peptone agar and were found to be indistinguishable. We concluded that all the single spore isolations of (D1) were alike.

DISCUSSION

It seemed to us that the concept of the "dual phenomenon," as we interpret its application by Wilhelm to dermatophytes, could be tested by selecting a number of variants which had developed in old cultures of the condial form of a "race" of *T. mentagrophytes*. Evidence on the existence of heterocaryosis and nuclear separation could be obtained by comparing cultures derived by single spore isolations from young cultures of these variants.

Since our strain of *T. mentagrophytes* was isolated from a human subject and developed from a single microconidium, we considered it to be a single "race." The variants were all obtained from this "race."

Some of the variants selected were intermediate between the ex-

treme mycelial type and the pronounced conidial form in growth habit, conidial production and microscopic appearance. We considered that at least some, if not all, of these would be intermediates in Wilhelm's meaning and therefore heterocaryotic. If this assumption were correct, then both the mycelial and conidial types would be obtained by single spore isolations from these intermediates. If the spores isolated were uninucleate, these two types only would be observed. If the spores or other reproductive bodies were multinucleate, a few of the conidial and a few of the mycelial types might be expected, but various kinds of intermediates, depending upon the relative proportions of C and M nuclei in the spores isolated, would predominate. Young cultures only could be used for single spore isolations because of the possibility that mutants had developed in the older cultures.

Our results were entirely negative. In no instance did we obtain a conidial or a mycelial type from the microconidia isolated from young cultures of forms which appeared to be intermediate between the two types. In every instance the cultures which grew from single microconidia were indistinguishable from the parent.

The number of conidia from each isolation with which we dealt was not large. We believe, however, that it was sufficiently large to detect nuclear separation, though too small to discover spontaneous mutants which might be assumed to occur at the rate of 1 to 10,000 or of that order.

It seems unlikely that Wilhelm's suggestion is adequate to explain the variants which develop from a single strain of *T. mentagrophytes*. If the variants with which we worked were derived from multinucleate spores, we should have observed nuclear separation. If they originated from spores which were uninucleate, they should have been either conidial or mycelial but not intermediate.

We are of the opinion that the variants which develop, as cultures of *T. mentagrophytes* age, are mutations, though whether the change is cytoplasmic or nuclear cannot be determined because of the absence of a sexual stage.

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THE SWARM–CELLS OF MYXOMYCETES

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(WITH 25 FIGURES)

Germination of spores of a species of myxomycete was first reported by deBary (1) in 1854. He observed that the spores of *Hemitrichia Vesparium* [1] produced a flagellated swarm-cell rather than the hyphal tube characteristic of the higher fungi, with which the Myxomycetes were then classified. Subsequently deBary (3, 4) attempted to germinate spores of a number of species, meeting with varying success. He obtained no germination of spores of the *Cribrarias* and the *Tubiferas*.

Since deBary's first report, many students of the Myxomycetes have attempted to germinate a large number of forms and have invariably met with the same inconsistencies which deBary found. Durand (13) attempted to germinate several species, succeeding only with *Enteridium Rozeanum*. McClatchie (43), reading of Durand's low results, replied with a statement that he and his students never failed to obtain good germination with spores of *Reticularia Lycoperdon* whether the collections were fresh or a year old. He germinated five other species with varying results, but always obtained swarm-cells within three hours except in *Hemitrichia Vesparium,* which produced only myxamoebae. Lister (40) found that repeated drying and rewetting of cultures facilitated the germination of a species of *Badhamia*. Jahn (29) used this method to obtain germination of *Stemonitis axifera* after previous culturing had failed, and later (30) used it to obtain germination—in very low numbers, to be sure—of *Tubifera ferruginosa* and *Lindbladia effusa*. Both of these belong to families with which deBary had had no success. All reports since Jahn's confirm deBary's experience with these two species. F. A. Gilbert (21) made a comparative study of spore germination by families.

[1] Nomenclature throughout this paper follows the classification according to Martin (42). Where current names differ from those used by the authors of the papers cited, the synonymy is given in the appendix.

He included reports in the literature as well as results obtained with his own collections of fifty-six species. He reports that all of the Reticulariaceae germinated very well, and that the *Tubiferas* had not ever been germinated, apparently overlooking Jahn's report. He found that in the other families tested "germinative ability appears to differ with the genera and species."

Studies of the factors influencing spore germination have brought out many points but have left many others to be clarified. McClatchie reported a higher percentage of germination in *Reticularia Lycoperdon* one year old than in fresh collections. Gilbert reported no germination of *Lycogala* less than one month after fruiting, but good germination of older material. He found that fresh collections of *Leocarpus fragilis* germinated only after two and a half days in culture while older spores germinated in twelve hours. E. C. Smith (**55**) germinated twenty-one collections ranging from five to thirty-two years old and found no correlation between the ages of his collections and their percentages of germination. Other workers have observed improvement of germination with aging within relatively short limits of time. Gilbert regarded the aging of the spores as important in their maturation and listed this as one of the most important factors governing germination. He also referred to "internal factors" varying from species to species.

Smart (**52**) studied the influence of some external factors on the germination of about seventy species. He found that most forms germinate better, though usually little better, in decoctions of their natural substrata than in distilled or tap water. The optimum temperature for all forms studied was between 22° and 30° C., most forms doing better near 30° C. than at lower temperatures. The optimum pH ranged from 4.5 to 7.0, most forms germinating most readily near neutrality. It is important to note that the variation in percentage germination of any given species resulting from variation of any one factor is less than the differences in percentage germination among the various species even though all factors be optimum for each species. In other words, Smart's intensive study of external factors serves to bring out the point that the factors he studied are of less importance in spore germination than some other factor or factors not yet discovered.

Several workers have stressed the necessity of stirring the spores

into the culture solution to assist in wetting. Cayley (9) used alcohol as a wetting agent to increase germination of spores of *Didymium nigripes*. Smart (52) disinfected spores with mercuric chloride or hexylresorcinol, followed by washing with centrifuging and final culturing in distilled water. Though his objective was reduction of bacteria, probably the manipulation had considerable wetting action, and many of the side effects which he observed might be attributable to increased numbers of spores thoroughly wetted.

Gilbert (18) and Smith (54) both reported that approximately half of the species which they observed produced more than one swarm-cell per spore. Gilbert observed the production of one, two, or four protoplasts from spores of *Leocarpus fragilis,* and Smith states that he observed four protoplasts escape from each of fifty spores of *Badhamia affinis* before seeing a single protoplast emerge from one spore. Howard (26) says spores of *Physarum polycephalum* may produce one or two swarm-cells, and Heitzmanowna (24) observed "one to several" swarm-cells produced by spores of *Didymium nigripes*.

Cultures of swarm-cells of Myxomycetes are ordinarily contaminated by bacteria, and usually by any or all of several species of protozoa. DeBary pictures a protozoan with a flagellum at each end among his swarm-cells. Durand observed this same form and considered it an abnormal form of swarm-cell characteristic of *Enteridium Rozeanum*. Pinoy (45, 46) considered certain bacteria and protozoa necessary for germination and development of Myxomycetes and even postulated that the protozoan cysts are incorporated into myxomycete fructifications. Gilbert studied the bipolar flagellate and reported it as *Cercomonas longicauda*.

Anteriorly biflagellate swarm-cells have been observed as occurring occasionally in Myxomycetes by many observers, but it has been generally held that the swarm-cells in this group are normally uniflagellate. DeBary (4) in 1884 and Vouk (58) in 1911 reported biflagellate forms in exceptional cases only. Gilbert (16) found one-fourth of the swarm-cells of *Stemonitis fusca* biflagellate. Von Stosch (56) saw biflagellate cells in many other species, but none in the single species of *Stemonitis* which he studied. Gilbert (17) reported *Dictydiaethalium plumbeum* to be uniflagel-

late, but E. C. Smith (**54, 55**) twice within the following year published photomicrographs showing biflagellate swarm-cells in the same species. Howard (**26**) termed biflagellate swarm-cells in *Physarum polycephalum* "common." Sinoto and Yuasa (**49**) studied *Ceratiomyxa* and four species of Myxogastres, finding only one flagellum in *Ceratiomyxa,* but occasional bi- and even tri-flagellate forms in all of the others. Yuasa (**60**) likewise found bi- and tri-flagellate cells in *Fuligo septica.* Jahn (**31**) in 1928 ignored the previous reports of biflagellate swarm-cells, and in 1936 (**32**) he criticized the work of Von Stosch, insisting that swarm-cells are normally uniflagellate and that all biflagellate swarm-cells are anomalies. Karling (**35**), in his general summary of the literature pertinent to the relationships between the Plasmodiophorales and the Myxomycetes, says, ". . . although the majority are uniflagellate, zoospores with two flagella are not uncommon. . . ." In 1945 Ellison (**15**) reported biflagellate swarm-cells in proportions varying from two per cent to twenty-six per cent for a number of species of Myxomycetes but retained the assumption that the majority are uniflagellate.

It is not inconceivable that an occasional biflagellate swarm-cell could occur as an abnormality in an otherwise uniflagellate group. But biflagellation has already been reported too frequently in the Myxomycetes to represent mere abnormality. And on the basis of flagellation as reported in other groups, it is very unlikely that both uniflagellate and biflagellate forms would normally exist in the same life stage of the Myxomycetes. It seems more probable that the second, shorter, flagellum is difficult to see and is frequently hidden.

The purposes of this study were to find a means of improving germination *in vitro* of myxomycete spores, and to study the swarm-cells thus produced in an effort to determine whether anterior biflagellation is not the rule rather than the exception.

METHODS AND MATERIALS

Ninety collections representing fifty-nine species in thirty genera were used for these tests. Following is a list of the collections used, with the place and year of collection.

Ceratiomyxales
  Ceratiomyxaceae
    Ceratiomyxa fruticulosa (Muell.) Macbr.

1.	Canal Zone	1945
2.	Minnesota	1946
3.	Iowa	1948

Liceales
  Liceaceae
    Tubifera ferruginosa (Batsch) J. F. Gmel.

4.	Iowa	1934
5.	Montana	1935
6.	Illinois	1947

  Reticulariaceae
    Dictydiaethalium plumbeum (Schum.) Rost.

7.	Iowa	1947
8.	Iowa	1947

    Enteridium Rozeanum (Rost.) Wing.

9.	Ohio	1893
10.	Iowa	1946
11.	Iowa	1946
12.	Iowa	1947

    Lycogala epidendrum (L.) Fries

13.	Indiana	1944
14.	Iowa	1946
15.	Iowa	1947

    Reticularia Lycoperdon Bull.

16.	Iowa	1929
17.	Iowa	1942
18.	New York	1947
19.	Iowa	1947

  Cribrariaceae
    Cribraria intricata Schrad.

20.	Nebraska	1894
21.	Illinois	1940

    Cribraria purpurea Schrad.
    Dictydium cancellatum (Batsch) Macbr.
    Lindbladia effusa (Ehrenb.) Rost.

22.	West Virginia	1947
23.	Iowa	1942
24.	South Dakota	1910

Trichiales
  Trichiaceae
    Arcyria cinerea (Bull.) Pers.
    Arcyria denudata (L.) Wettst.
    Arcyria incarnata Pers.
    Hemitrichia clavata (Pers.) Rost.
    Hemitrichia Serpula (Scop.) Rost.
    Hemitrichia stipitata (Massee) Macbr.
    Hemitrichia Vesparium (Batsch) Macbr.
    Oligonema Schwenitzii (Berk.) Martin

25.	Canal Zone	1945
26.	Iowa	1947
27.	Iowa	1946
28.	Iowa	1946
29.	Kentucky	1945
30.	Kentucky	1946
31.	West Virginia	1947
32.	Iowa	1947
33.	Iowa	1947
34.	Iowa	1947

    Perichaena chrysosperma (Currey) Lister
    Perichaena depressa Libert
    Trichia affinis deBary
    Trichia favoginea (Batsch) Pers.
    Trichia varia (Pers.) Pers.

35.	Kentucky	1947
36.	Georgia about	1940
37.	Iowa	1946
38.	Iowa	1947
39.	California	1947

Stemonitales
  Echinosteliaceae
    Echinostelium minutum deBary

40.	Louisiana	1936
41.	Iowa	1937
42.	Iowa	1943
43.	Indiana	1945
44.	Minnesota	1946
45.	Rhode Island	1947

  Stemonitaceae

Clastoderma Debaryanum Blytt	46.	Rhode Island	1947
Comatricha laxa Rost.	47.	Iowa	1945
Comatricha Suksdorfii Ellis & Ev.	48.	California	1947
Comatricha typhoides (Bull.) Rost.	49.	Iowa	1937
	50.	Minnesota	1947
Diachaea bulbillosa (Berk. & Br.) A. Lister	51.	Canal Zone	1945
Enerthenema melanospermum Macbride & Martin	52.	California	1946
Enerthenema papillatum (Pers.) Rost.	53.	Indiana	1945
	54.	Illinois	1947
Lamproderma arcyrionema Rost.	55.	Illinois	1947
Lamproderma Sauteri Rost.	56.	California	1946
Lamproderma scintillans (Berk. & Br.) Morgan	57.	Iowa	1942
Stemonitis axifera (Bull.) Macbr.	58.	Maryland	1894
	59.	Michigan	1935
	60.	Indiana	1944
	61.	Kentucky	1946
	62.	California	1947
Stemonitis flavogenita Jahn	63.	West Virginia	1947
Stemonitis fusca Roth.	64.	Iowa	1927
Stemonitis splendens Rost.	65.	Iowa	1947

Physarales
  Physaraceae

Badhamia gracilis Macbr.	66.	Panama	1935
Badhamia panicea (Fries) Rost.	67.	Iowa	1938
Cienkowskia reticulata (Alb. & Schw.) Rost.	68.	Louisiana	1948
Craterium leucocephalum (Pers.) Ditm.	69.	Iowa	1941
	70.	Illinois	1947
Fuligo septica (L.) Wiggers	71.	Indiana	1944
	72.	Michigan	1947
Leocarpus fragilis (Dicks.) Rost.	73.	Iowa	1946
Physarella oblonga (Berk. & Curt.) Morgan	74.	Kentucky	1947
Physarum cinereum (Batsch) Pers.	75.	Iowa	1939
Physarum contextum (Pers.) Pers.	76.	Illinois	1947
Physarum didermoides (Pers.) Rost.	77.	Illinois	1947
Physarum flavicomum Berk.	78.	Pennsylvania	1886
Physarum globuliferum (Bull.) Pers.	79.	Iowa	1946
Physarum melleum (Berk. & Br.) Massee	80.	Hawaii	1946
Physarum nutans Pers.	81.	Iowa	1942
Physarum polycephalum Schw.	82.	Iowa	1947
Physarum viride (Bull.) Pers.	83.	Rhode Island	1945

Didymiaceae

Diderma floriforme (Bull.) Pers.	84. Iowa	1947
Diderma globosum Pers.	85. Iowa	1947
Didymium squamulosum (Alb. & Schw.) Fries	86. Minnesota	1946
	87. Iowa	1947
	88. Iowa	1948
Didymium xanthopus (Ditm.) Fries	89. Iowa	1948
Mucilago spongiosa (Leysser) Morgan	90. Indiana	1944

The first cultures were prepared in September, 1946, using *Reticularia Lycoperdon* (Coll. no. 16, above), *Lycogala epidendrum* (no. 14), and *Trichia affinis* (no. 37). Abundant germination was obtained in the first attempts with *R. Lycoperdon* and *T. affinis,* but only one of several cultures of *L. epidendrum* was observed to germinate. Repeated attempts to germinate cultures of the two oldest collections of *Enteridium Rozeanum* (nos. 10 and 11) resulted in the observation of occasional swarm-cells in no. 10, but only very low percentages of germination. Other species gave similar results.

Cultures were made in Syracuse watch glasses using distilled water from which the traces of toxic minerals were removed with powdered charcoal. It was observed that most of the spores placed in the culture dish continued to float on the surface of the water, never becoming wet. This was especially true of those forms having very small spores, such as *Enteridium* and *Lycogala*. Various wetting agents were tried in an attempt to accelerate the wetting and increase the germination of these spores.

Alcohol was the first wetting agent tested. Cayley (9) used 20% alcohol for wetting spores of *Didymium* sp., securing approximately 50% germination whether the spores were in the alcohol "a few minutes" or a full hour. Also she used a solution of 0.2% mercuric chloride in a mixture of equal parts of 95% alcohol and water, as a combined wetting agent and bactericide. The resulting cultures were not bacteria-free and germination was poor.

In the tests here reported a number of different dilutions of alcohol were tried, 95% being the strongest and 20% the weakest concentration used. Lower concentrations had negligible wetting effect. After wetting, spores were washed three times with centrifuging and cultured as before. No germination was obtained
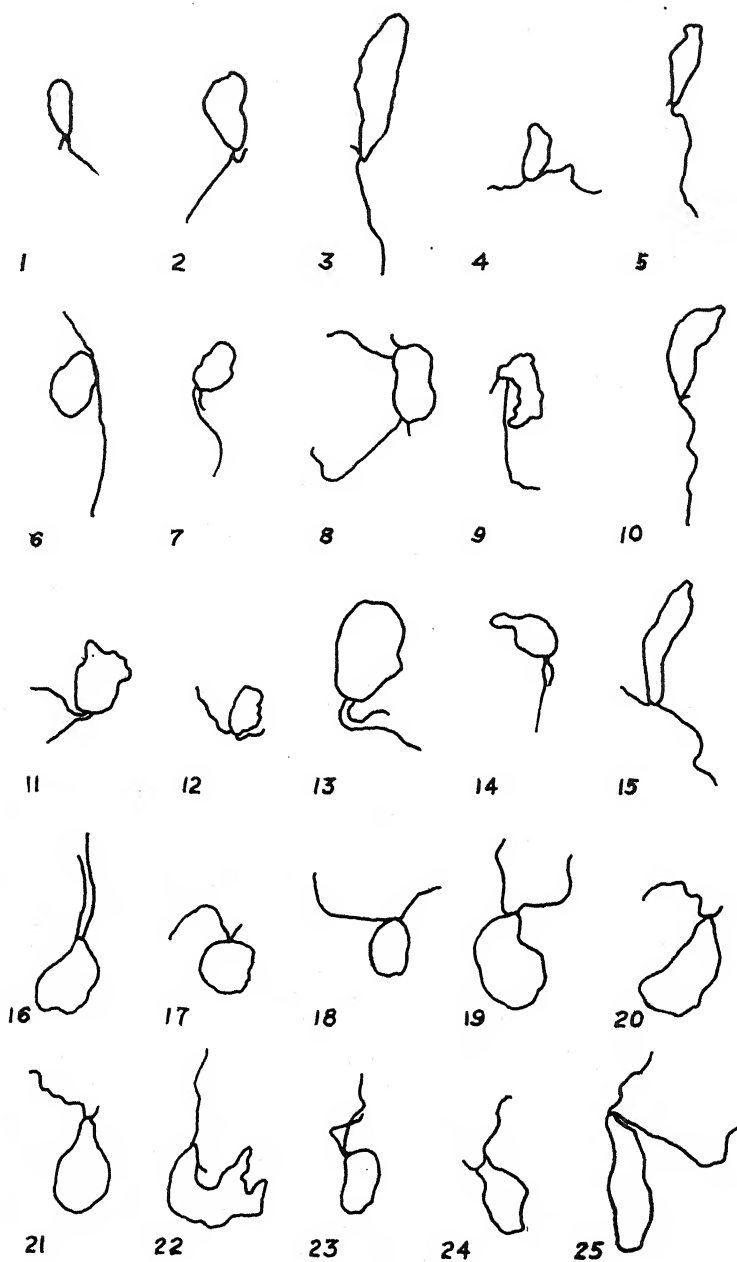
Drawings of Figs. 1–25.
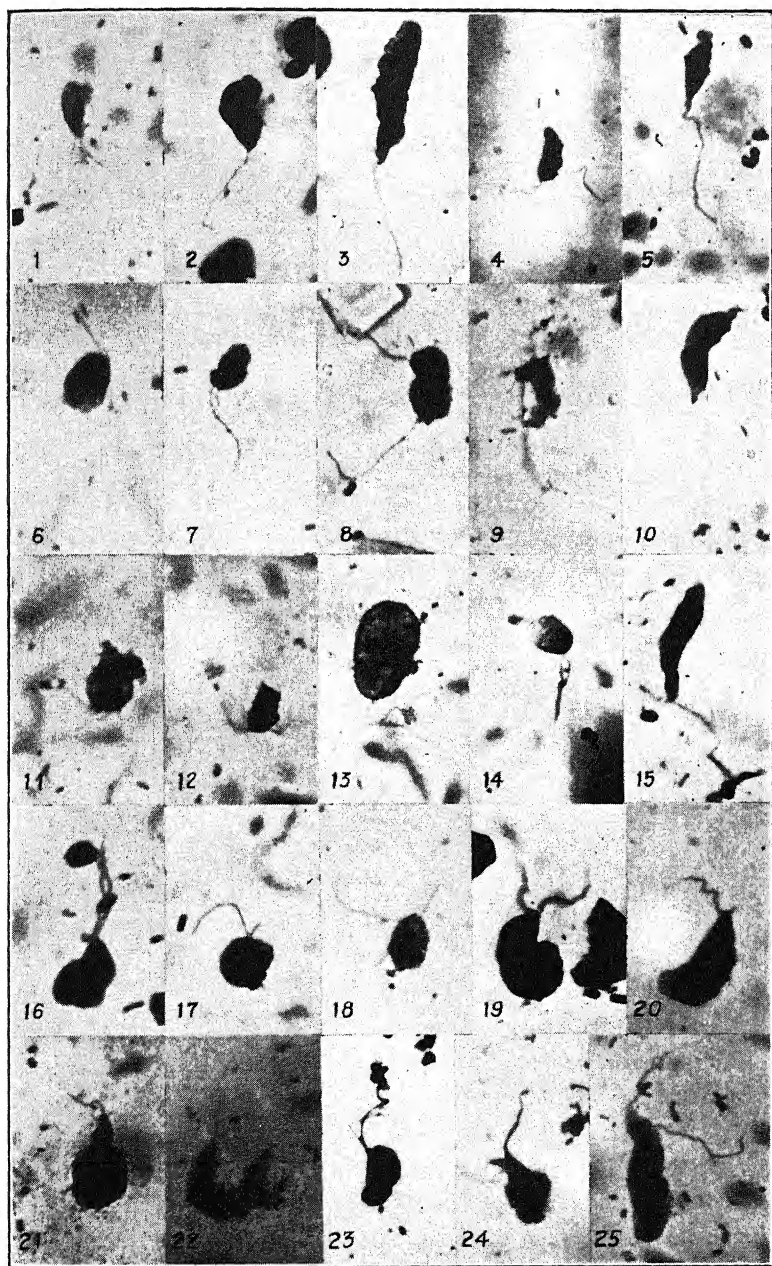
FIGS. 1–25. Swarm cells of Myxomycetes.

from spores wetted with alcohol, even in *Reticularia Lycoperdon,* in which nearly 100% germination had been secured without the use of a wetting agent.

Trisodium phosphate was tried next. By experimentation it was found that spores of *Enteridium Rozeanum,* which has the smallest spores of any species used up to the time of these tests, would sink immediately in a 0.5% solution and slowly in a 0.2% solution. In preparing cultures using trisodium phosphate as a detergent, washing was done as when alcohol was the wetting agent. Cultures of *E. Rozeanum* (no. 11), prepared with the use of trisodium phosphate in either 0.5% or 0.2% solution, germinated nearly 100% within one hour. Swarm-cells had not been seen in cultures of this collection before.

Trisodium phosphate in 0.5% solution was used as a detergent in preparation of cultures of a number of other collections. Abundant germination was produced occasionally in *Lycogala epidendrum* (no. 14), but no consistent germination was secured with any species except *E. Rozeanum;* hence toxic effects were suspected.

In the search for an active detergent without toxic effects, two commercial detergents, "Soilax" and "Dreft," were tried, both in 0.5% solution. Both acted as effective wetting agents, but many spores were caught in the foam on the "Dreft" solution and could not be reclaimed by centrifuging. Germination in cultures thus prepared was similar to, and no more satisfactory than, that obtained with trisodium phosphate.

The fact that bile salts greatly lower the surface tension of solutions, as exemplified in the Hay test for bile in urine (23), inspired the testing of solutions of sodium glycocholate and sodium taurocholate as wetting agents for preparation of cultures. It was found that either the glycocholate or the taurocholate will wet the spores satisfactorily in 1% solution, but lower concentrations act so slowly as to be inadequate. In equal concentrations, these two salts, or a mixture of them, are equally effective as wetting agents.

Spores of many species of Myxomycetes which did not germinate at all when prepared directly in water or with other detergents, germinated when previously wetted with either of the bile salts. And all those collections which germinated directly in water germi-

nated more quickly and in greater percentage when previously wetted with the bile salts. Of course, the spores of two different collections of the same species frequently show greatly different percentages of germination. When the bile salts are used, other conditions being equal, the differences are substantially reduced.

Germination of spores directly in 1% solution of the bile salts was tried. Protoplasts emerged from the spore cases, but developed no further. However a technique was developed in which only one washing with water is necessary to free wetted spores of the detergent. Approximately 1 cc. of the wetting agent is placed in a centrifuge tube and the spores to be cultured are added and stirred until wetted, a process which usually takes one-half minute. Then the solution is diluted to 5 or 6 cc. with water and promptly centrifuged. The spores are then washed once with distilled water by centrifuging, and are finally cultured in distilled water prepared with charcoal as described above.

The wetting efficiency and the low toxicity of the "Aerosols" recommend them for the purpose of preparing cultures of myxomycete spores. It was hoped that the toxicity of an aerosol solution would be negligible and that a dilution might be found which would be effective in wetting and of such a nature that germination could take place directly in the detergent. As before, spores of *Enteridium Rozeanum* were used as the test organism, and it was found that considerable wetting effect was obtained in solutions as weak as 0.1 ppm. Germination was obtained in solutions as strong as 1000 ppm., the highest percentage of germination in these tests being at 1 ppm. However, when *Reticularia Lycoperdon* was tested in the same dilutions, no germination was obtained in solutions stronger than 1 ppm., and very few swarm-cells were found in solutions stronger than 0.1 ppm. No germination was obtained in aerosol solutions with any of several other forms which were tried, though germination was obtained with all of them in cultures prepared at the same time using the bile salts as described above. Many protoplasts, escaped from the spore cases, were observed to disintegrate explosively in solutions stronger than 1 ppm. in all cultures except those of *Enteridium Rozeanum*. However, germination was obtained in the two forms mentioned above, and the

swarm-cells remained alive in the flagellated form for much longer than was ever observed in cultures prepared otherwise. It is concluded, therefore, that the chemical toxicity of the aerosols is not an important factor, but that the surface tension of the culture solution is of considerable importance. Probably a solution of aerosol could have been used in place of the bile salts solution, with the same washing technique. But since washing would obviate the only advantage of using aerosol, the bile salts were continued, for the sake of uniformity of results.

Alternate wetting and drying, tried on several cultures, gave no noticeable improvement in any case except that of *Physarum flavicomum*, the oldest collection germinated. This culture, prepared by the usual method, germinated in very low numbers. Dried overnight and rewet, it germinated in very high percentage in about six hours.

It was found that in order to preserve the flagella of swarm-cells for observation, the killing agent used in preparation of material for microscopic examination must act very quickly. Smears prepared by air-drying, or even by drying as quickly as possible over mild heat, as is done in the preparation of bacterial mounts, showed recognizable swarm-cells, all of which, however, completely lacked flagella.

The most satisfactory results for temporary mounts were obtained by killing and staining on the slide with a drop of iodine-potassium iodide solution. For this purpose Gram's iodine is used without dilution, density of staining being controlled by varying the proportions of culture solution and iodine solution which are mixed on the slide. Whatever proportions are used, mixing must be accomplished quickly and thoroughly if the flagella are to be preserved for observation.

Permanent slides were prepared for observation of flagella by use of a modification of the Loeffler stain for bacterial flagella (6). The mordant and stain were prepared as directed, but the times of application of both mordant and stain were reduced from five minutes to one-half minute. Swarm-cells thus stained with carbol fuchsin were destained to transparency with acid alcohol, counter-

stained with fast green in clove oil and mounted in clarite.   On a properly prepared slide the cell body is greenish, the nucleus red, and the flagella deep green to purplish.

Smears were prepared by various means.   Slides were thinly coated with albumin fixative, on which a drop of culture solution was placed and inverted over osmic acid fumes.   This was then allowed to dry in air.   Other smears, killed over osmic acid, were fixed by heating gently after drying.   Still others were killed with iodine, as was done in the preparation of temporary mounts, and allowed to stand until the iodine had sublimed.   These slides were fixed over heat.   The crystals of potassium iodide were dissolved off in distilled water before staining.   Equally satisfactory results were obtained with all these methods.   The iodine method, being the simplest, was used.

RESULTS AND DISCUSSION

Germination.   Cultures were prepared, by the method described above, of 90 collections of Myxomycetes.   All orders, all families except the Dianemaceae and Collodermataceae, 30 genera, and 59 species were represented.   Germination was obtained in 80 of the collections.   At least one culture of each species except *Echinostelium minutum* produced swarm-cells.   Following is a list of collections in which swarm-cells were not observed:

21.	*Cribraria intricata*	1940
40–45.	*Echinostelium minutum*	1936–1947
61.	*Stemonitis axifera*	1946
69.	*Craterium leucocephalum*	1941
87.	*Didymium squamulosum*	1947

E. minutum is such a small form that no collection provided sufficient material to be handled as much as is required for wetting by the bile salts method.   Cultures had to be prepared directly in water, in hanging drop mounts.   It may be that these spores never were adequately wetted, or it may be that fewness of spores resulted in a lack of the mass-action factor postulated by Wilson and Cadman (**59**) in the case of *Reticularia Lycoperdon*.

It has long been recognized that each collection, rather than

each species, is a problem in itself. The bile salts technique for the wetting of spores solved many problems in the way of germination, favorable results being achieved in most cases. But advance wetting before culturing could not be expected to overcome all the "internal factors" referred to in the literature, and so failure was met in the four collections listed above, others of the same species germinating well.

That wetting of spores is an important factor in germination *in vitro* is obvious; for many collections which would not germinate at all when cultured directly in water germinated readily when previously wetted, and forms which germinated in very low numbers when cultured directly in water produced a higher percentage of swarm-cells when wetted before culturing.

Age of the collection seems to have no important bearing on germination within the limits of these experiments. In general, a higher percentage of germination was obtained with material less than a year old than with older material of the same species. However, Gilbert's observation that spores of *Lycogala epidendrum* do not germinate until the fructification is a month old was confirmed. Collection no. 15 was cultured on the day after collection, and each week thereafter, each culture being maintained for one week. No swarm-cells were observed until the fifth culture, made four weeks after collection, which germinated abundantly in about six hours.

Smith germinated spores of collections as much as 32 years old, finding no correlation between age and percentage of germination. In this study were included five collections more than 32 years old:

9.	*Enteridium Rozeanum*	1893
20.	*Cribraria intricata*	1894
24.	*Lindbladia effusa*	1910
58.	*Stemonitis axifera*	1894
78.	*Physarum flavicomum*	1886

Again, no correlation was found between age and percentage of germination. The oldest collection was cultured simultaneously with 20 other collections of 19 different species, the ages ranging from a few months to about 10 years. Swarm-cells were observed in *Physarum flavicomum* before half of the others germinated. As

is mentioned above, germination in this very old collection was markedly improved by drying and rewetting the culture. None of the other 20 collections responded to this treatment, and so within six hours after rewetting the percentage of germination in *P. flavicomum* was much higher than was ever found in any of the rest. The *Stemonitis axifera* was cultured with three others of the same species, ranging from about one year to 13 years. The freshest culture germinated 24 hours ahead of the rest, but percentage of germination was about equal in all four, and apparent vigor of the swarm-cells was equal in all cultures.

Seventeen species not listed as previously germinated were germinated in these tests. Two monotypic genera are represented: *Clastoderma Debaryanum* and *Cienkowskia reticulata.* Kambly (**34**) reports that attempts to produce plasmodia of *C. Debaryanum* from spores were unsuccessful, but it is not clear whether he observed germination of spores and production of swarm-cells of this species. He further states that the plasmodium of *C. Debaryanum* would not grow in artificial media away from its natural substratum. No special care was given the spores in these tests, but swarm-cells were produced in abundance.

Emergence of protoplasts from spore cases was observed in every culture of every form in which germination occurred. In no case was a flagellum observed on a protoplast at the time of emergence. In these cultures, flagella were formed only after a resting period of varying duration, and in several species smears made at frequent intervals after emergence was first observed, showed the growth of the flagellum to take place at a rather slow rate, requiring as much as two hours in some cultures.

In no culture was the emergence of more than one protoplast from a single spore observed. All of the swarm-cells of *Trichia affinis* and of *Physarum melleum,* and most of those of *Trichia favoginea,* which were observed, were smaller than would be expected if a single swarm-cell were produced by each spore. But the protoplasts seen emerging were the full content of the spore, and so it is assumed that the size of the swarm-cells is the result of divisions outside the spore case.

No germination was obtained at any time if the room tempera-

ture was above 32° or 33° C.   For this reason, experimental work
had to be discontinued during the hotter days of the summer.
Also, it was observed that whenever germination did take place
near the upper limit of tolerance, more myxamoebae and fewer
swarm-cells were found in the cultures than when cultures of the
same collections germinated at temperatures nearer 25° C.

As is stated above, it is believed that the surface tension of the
culture solution is of considerable importance in the germination of
myxomycete spores.   Unfavorable response to the low surface ten-
sion is the only obvious explanation of the reaction to aerosol solu-
tions observed.   The fact that no germination, even of *Enteridium
Rozeanum,* was obtained in the bile salts solution without washing
may be interpreted as implying that the bile salts are toxic, whether
the surface tension of the solution is unfavorable or not.   All ob-
servers report best germination in neutral or slightly acid media.
The apparent toxicity of trisodium phosphate and the commercial
detergents is probably due to their alkalinity.

Every culture prepared was observed to be contaminated with
bacteria from the time of preparation, and with various protozoa
if maintained more than 24 hours.   Ciliates of the genus *Colpoda*
and a flagellate identified as *Cercobodo agilis* were the most com-
mon.   The flagellate is of approximately the same size as a myxo-
mycete swarm-cell, but moves with a gliding motion so different
from a swarm-cell that the two forms are not easily confused even
under the lowest powers of the microscope.   *Cercobodo agilis* has
one anterior flagellum and a second flagellum equal to the first
arising near the middle of the body.   The nucleus is approxi-
mately central, and the second flagellum arises near the nucleus.
This flagellum always trails and appears to be posterior, but that it
is not attached posteriorly is easily seen on living mounts as well
as on permanent, stained slides if high magnification is used.   The
bipolar flagellate frequently reported in the literature was not seen
in any culture.   The flagellate here described occurred in nearly
every culture, was most numerous in cultures of *Ceratiomyxa,* and
flourished and encysted prior to observation of swarm-cells in all
cultures of all collections more than 20 years old.

Flagellation.   Some biflagellate swarm-cells were observed in

every species studied.   In several species the proportion of swarm-cells obviously biflagellate was nearly 100%. *Lycogala epiden-drum, Cribraria purpurea* (FIG. 3), *Oligonema Schweinitzii* (FIG. 7), *Leocarpus fragilis* (FIG. 18), *Fuligo septica* (FIG. 17), and *Physarella oblonga* (FIGS. 19, 20) were the most notable among these.   In most species the second flagellum is usually difficult to see, but with careful, experienced observation it can be found on nearly every swarm-cell studied.   Zygotes with four flagella, representing two biflagellate swarm-cells fused posteriorly, were found occasionally in *Dictydiaethalium plumbeum, Cribraria purpurea, Oligonema Schweinitzii* (FIG. 8), *Stemonitis fusca,* and *Tubifera ferruginosa.*

On a great majority of swarm-cells of most species, the second flagellum is very short, scarcely more than 1 μ long.   (Swarm-cells in this group will be referred to as "type I.")   See figures 1, 4, 5, and 10.   Other swarm-cells have the second flagellum somewhat longer, measuring approximately half the length of the longer flagellum (type II), as shown in figures 12, 14, 15, and 18.   And on a few individuals the two flagella are nearly equal (type III), as shown in figures 3, 20, 23, and 25.   Previous accounts, though maintaining the assumption that swarm-cells of Myxomycetes are characteristically uniflagellate, have brought out this variation in the length of the second flagellum.   E. C. Smith (**54, 55**) published photomicrographs showing biflagellate swarm-cells of *Reticularia Lycoperdon, Dictydiaethalium plumbeum, Lepidoderma tigrinum,* and *Physarum cinereum* with the second flagellum distinct, and *Badhamia orbiculata* in which only a dark line on the body of the cell is seen.   All of the swarm-cells in his pictures are type I.   Gilbert (**16**) showed *Stemonitis fusca* with two flagella nearly equal, though the rest of the forms he drew were definitely heterocont.   Ellison (**15**) drew swarm-cells of *Stemonitis fusca* and *S. axifera* with two flagella nearly equal even though the others he records have one flagellum much shorter than the other.   (*Cf.* FIGS. 14, 15, and 16.)

In a previous report on this same investigation (**14**) it was stated that all swarm-cells of *Stemonitis splendens* are of type III,

but all other species studied up to that time have swarm-cells of type I. It was further stated that the ratio of the lengths of the two flagella is quite constant in any given species. Further study has revealed exceptions to these generalizations. Some smears of *Stemonitis splendens* have contained numerous swarm-cells with type II flagellation. None of type I, however, have been found in this species. More variation in the length of the second flagellum is found in those species in which it is usually of type II or type III than in those in which it is shorter. However, smears of *Physarella oblonga* contain about 10% swarm-cells of type III, the rest being of type I. None of type II was found. (*Cf.* FIGS. 19 and 20.)

All swarm-cells of species of the Reticulariaceae and of the Trichiaceae which were studied were of type I, and all swarm-cells of *Tubifera ferruginosa,* the only member of the Liceaceae included in this investigation, were also of type I. In the Physaraceae most of the swarm-cells of the larger genera are of type I, and most of the swarm-cells of the remaining genera are of the longer types; but there is some variation in most species. Most members of the Cribrariaceae, Stemonitaceae, and Didymiaceae have swarm-cells predominantly of types II and III, though here again, there is considerable variation within most species.

The second flagellum, in addition to being very short, is usually recurved so as to be almost indistinguishable from the outline of the cell itself (FIG. 21). Even in those species in which both flagella are relatively long, one is usually trailing, so that it is easily overlooked (FIG. 6). The significance of this point is borne out in the following observation: A temporary mount was prepared from a culture of swarm-cells of *Dictydiaethalium plumbeum*. The mount was killed and stained with Gram's iodine. Using the 90 × oil immersion objective, the microscope was focused upon a swarm-cell in which both flagella were easily seen. Then, by touching the edge of the cover slip with a dissecting needle, the mount was disturbed while the original swarm-cell was kept in view. This swarm-cell was observed to roll over and again come to rest. In its new position, the shorter flagellum could not be seen with any manipulation of the microscope. A second swarm-cell which pre-

sented only the longer flagellum to view was found, and by similarly disturbing the cover slip the swarm-cell was maneuvered until the shorter flagellum could also be seen. This same demonstration of the fact that the shorter flagellum may be—and frequently is—hidden by the body of the cell, was also performed on mounts of *Fuligo septica* and *Arcyria denudata*.

The second flagellum is clearly visible on only a very few swarm-cells in most mounts. Some mounts stained with Loeffler's technique were destained to transparency with acid alcohol. On swarm-cells thus prepared the flagella remained clearly stained, but the body of the cell was sufficiently destained so that the nucleus and other cellular details were visible. When swarm-cells are properly stained by this method, careful focusing on the anterior portion of the body of the cell will reveal a dark line reaching backward from the apex to the base of the conical anterior portion. Occasionally this dark line will be found lying across the conical portion, either on or underneath the body of the cell, but most frequently it is barely distinguishable from the outline of the cell. It is not found on those swarm-cells on which the second flagellum is clearly visible apart from the body of the cell. It is believed that this is the shorter flagellum which ordinarily is closely appressed to the anterior portion of the cell. Figures 5, 10, and 21 illustrate this condition to some extent, but the cases in which the second flagellum is most difficult to see could not, of course, be photographed.

CONCLUSIONS

Spores of 80 collections representing 58 species of Myxomycetes were germinated. Germination was induced or improved by previous wetting, using a solution of the bile salts as a wetting agent. The flagellation of the swarm-cells of these species was observed in temporary and permanent preparations. In some species it was possible to see two flagella on nearly every swarm-cell observed. In all other species, two flagella were clearly visible on some swarm-cells, and it was demonstrated by manipulation of fluid mounts that the second flagellum could be brought into view even though not originally visible. From this it is inferred that if ade-

quate technique were used, the second flagellum would be found on all myxomycete swarm-cells.

Most of the biflagellate swarm-cells observed in this study were definitely heterocont. In most species the two flagella differ greatly in length. Even in those swarm-cells on which the two flagella are nearly equal, there is usually a distinct, though slight, difference in length.

Groupings of species based on the type of flagellation usually found on swarm-cells of each species show little correlation with the currently accepted classification of the Myxomycetes based on morphology of the fructifications. This, and the fact that there is considerable variation in the length of the second flagellum within some species, seems to make the taxonomic significance of the second flagellum, within the Myxomycetes, doubtful.

The Myxomycetes and the Plasmodiophorales have long been considered related groups by many investigators. Formerly, the reported existence of anteriorly uniflagellate zoospores in the reproductive cycle of both groups was regarded as strong evidence of this relationship. However, Ledingham (36, 37, 38) showed that the zoospores of the Plasmodiophorales are anteriorly biflagellate, the second flagellum being very short and hence easily obscured. As a result of Ledingham's finding, the supposed difference in flagellation was thought by many to emphasize a separation between the two groups.

The existence of two blepharoplasts has been reported in several species of Myxomycetes (16, 22, 56). Bessey (5) regards the second blepharoplast as a vestige of the biflagellate condition, indicating that the Myxomycetes and the Plasmodiophorales have arisen from a common ancestor. He considers the loss of the second flagellum as evidence that the Myxomycetes are of higher phylogenetic position.

Insofar as flagellation is of phylogenetic significance, the existence of the second flagellum in the swarm-cells of Myxomycetes, as demonstrated by this study, may indicate a closer relationship with the Plasmodiophorales than has recently been supposed.

This work was done in the Mycological Laboratory of the State University of Iowa under the direction of Professor G. W. Martin.

APPENDIX

CHECK LIST OF MYXOMYCETES PREVIOUSLY GERMINATED

In each instance in which the currently accepted name of a species differs from that used by an author cited, the synonym is given, indented in the first column, opposite the name of the author.

Species	Worker	References
Amaurochaete fuliginosa (Sow.) Macbr.		
as *A. atra*	Lister	39, 40
	Constantineanu	11
as *A. atra*	Jahn	29
as *A. atra*	Schunemann	48
Arcyria cinerea (Bull.) Pers.	F. A. Gilbert	21
	Smart	52, 53
as *A. digitata*	Smart	52, 53
Arcyria denudata (L.) Wettst.		
as *A. punicea*	deBary	3
	F. A. Gilbert	19, 20, 21
	Smart	52, 53
Arcyria ferruginea Sauter	Pinoy	45
Arcyria incarnata Pers.	Hoffmann	25
	Constantineanu	11
	F. A. Gilbert	19, 20, 21
	Smart	52, 53
Arcyria nutans (Bull.) Grev.	F. A. Gilbert	21
	Smart	52, 53
Arcyria Oerstedtii Rost.	F. A. Gilbert	20, 21
	Smart	52, 53
Arcyria pomiformis (Leers) Rost.	F. A. Gilbert	21
	Smart	52, 53
Badhamia affinis Rost.	Smith	54
Badhamia capsulifera (Bull.) Berk.		
as *B. hyalina*	McClatchie	43
Badhamia Curtisii (Berk.) Rost.		
as *B. rubiginosa*	F. A. Gilbert	21
as *B. rubiginosa*	Smith	54
as *B. rubiginosa*	Smart	52, 53
as *Physarum rubiginosum*	Smart	52, 53
Badhamia lilacina (Fries) Rost.	F. A. Gilbert	19, 20, 21
	Smith	54
	Smart	52, 53
Badhamia macrocarpa (Ces.) Rost.		
as *Physarum macrocarpum*	Hoffmann	3
	Constantineanu	11
	Jahn	29
Badhamia ovispora Racib.	Smart	52, 53
Badhamia panicea (Fries) Rost.	Smith	54, 55

Species	Worker	References
Badhamia utricularis (Bull.) Berk.	Lister	39
as *B. magna*	F. A. Gilbert	19, 20, 21
	Smith	54, 55
as *B. magna*	Smith	54
	Smart	52, 53
as *B. magna*	Smart	52, 53
Ceratiomyxa fruticulosa (Muell.) Macbr.		
as *Ceratium* spp.	Famintzin &	
	Woronin	41
	Olive	44
	Jahn	32
	Sinoto & Yuasa	49
	H. C. Gilbert	22
	Smart	52, 53
Comatricha elegans (Racib.) Lister	Smart	52, 53
Comatricha irregularis Rex	F. A. Gilbert	21
Comatricha laxa Rost.	Smart	52, 53
Comatricha longa Peck		
as *C. longa* var. *flaccida* Minakata	Sinoto & Yuasa	49
Comatricha nigra (Pers.) Schroet.		
as *Stemonitis obtusata*	deBary	3
	Smart	52, 53
Comatricha pulchella (Bab.) Rost.	Smart	52, 53
Comatricha typhoides (Bull.) Rost.	F. A. Gilbert	19, 21
Cornuvia Serpula Rost.		
as *Arcyria anomala*	deBary	3
Craterium leucocephalum (Pers.) Ditm.	Smith	54
	Smart	52, 53
Cribraria aurantiaca Schrad.		
as *C. vulgaris* var. *aurantiaca*	Constantineanu	11
	Smart	52, 53
Cribraria elegans Berk. & Curt.	Smart	52, 53
Cribraria intricata Schrad.	Smart	52, 53
Cribraria minutissima Schw.	Smart	52, 53
Cribraria tenella Schrad.	F. A. Gilbert	21
	Smart	52, 53
Diachaea leucopodia (Bull.) Rost.	McClatchie	43
	Smith	55
	Smart	52, 53
Dictydiaethalium plumbeum (Schum.) Rost.	F. A. Gilbert	17, 19, 20, 21
	Smith	55
	Smart	52, 53
Dictydium cancellatum (Batsch) Macbr.	Constantineanu	11
	F. A. Gilbert	20
as *D. cancellatum* var. *purpureum*	Sinoto & Yuasa	49
	Smart	52, 53
as *D. cancellatum* var. *purpureum*	Smart	52, 53
Diderma effusum (Schw.) Morgan	Smith	54

Species	Worker	References
Diderma globosum Pers.	Smart	52, 53
Diderma radiatum (L.) Morgan	Smith	54
Diderma testaceum (Schrad.) Pers.	Smart	52, 53
Didymium difforme (Pers.) S. F. Gray		
as *D. Libertianum*	deBary	3
as *Physarum album*	Cienkowski	10
	Lister	40
as *D. difforme* var. *comatum*	Lister	40
	Schunemann	48
	Jahn	29
	Skupienski	50, 51
	Cayley	9
Didymium melanospermum (Pers.) Macbr.	F. A. Gilbert	21
	Smith	54
	Schunemann	48
	Smart	52, 53
Didymium nigripes (Link) Fries	Pinoy	47
	Heitzmanowna	24
	Schunemann	48
	Cadman	7
	Abe	33
	von Stosch	56
	Kambly	34
Didymium Serpula Fries	Cienkowski	10
Didymium squamulosum (Alb. & Schw.) Fries		
as *Physarum Tussilaginis*	Kent	39
as *Didymium praecox*	deBary	3
as *Didymium effusum*	Pinoy	45, 46
	Constantineanu	11
as *Didymium effusum*	Jahn	30
	F. A. Gilbert	21
	Smith	54, 55
	Schunemann	48
	Smart	52, 53
	Kambly	34
Didymium xanthopus (Ditm.) Fries		
as *D. nigripes* var. *xanthopus*	F. A. Gilbert	19, 20, 21
as *D. nigripes* var. *xanthopus*	Cayley	9
	von Stosch	56
as *D. nigripes* var. *xanthopus*	Smart	52, 53
	Kambly	34
Enerthenema papillatum (Pers.) Rost.	Smith	54
Enteridium olivaceum Ehrenb.	Smith	55
Enteridium Rozeanum (Rost.) Wing.	Durand	13
	Jahn	29
	F. A. Gilbert	16, 19, 20, 21
	Smart	52, 53
Erionema aureum Penzig	Abe	33

Species	Worker	References
Fuligo septica (L.) Wiggers		
as *Aethalium septicum*	deBary	3
	McClatchie	43
as *Aethalium septicum*	Constantineanu	11
	F. A. Gilbert	18, 19, 20
	Cook & Holt	12
	Smith	55
	Abe	33
	Yuasa	60
	Smart	52, 53
	Kambly	34
	Ellison	15
Hemitrichia clavata (Pers.) Rost.	F. A. Gilbert	16, 19, 21
	Smith	54, 55
	Smart	52, 53
Hemitrichia Serpula (Scop.) Rost.	F. A. Gilbert	21
	Smith	54
	Smart	52, 53
Hemitrichia stipata (Schw.) Macbr.		
as *Arcyria stipata*	F. A. Gilbert	20, 21
Hemitrichia Vesparium (Batsch) Macbr.		
as *Trichia rubiformis*	deBary	1, 3
as *Trichia pyriformis*	deBary	3
as *Hemiarcyria rubiformis*	McClatchie	43
	F. A. Gilbert	19, 20, 21
	Smith	54
	Smart	52, 53
Lamproderma arcyriodes (Somm.) Rost.		
as *L. violaceum*	Smith	54, 55
Lamproderma arcyrionema Rost.	Smart	52, 53
Lamproderma columbinum (Pers.) Rost.	F. A. Gilbert	21
Leocarpus fragilis (Dicks.) Rost.		
as *L. vernicosus*	Hoffmann	25
	Constantineanu	11
	F. A. Gilbert	18, 19
	Schunemann	48
	Smart	52, 53
Lepidoderma tigrinum (Schrad.) Rost.	Smith	54, 55
Lindbladia effusa (Ehrenb.) Rost.	Jahn	30
Lycogala epidendrum (L.) Fries	deBary	3
as *L. miniatum*	Constantineanu	11
	F. A. Gilbert	19, 20, 21
	Cook & Holt	12
	Smart	52, 53
Lycogala flavo-fuscum (Ehrenb.) Rost.	F. A. Gilbert	20, 21
	Smith	54
as *L. flavo-fuscum* var. *solida*	Smith	54
	Smart	52, 53

Species	Worker	References
Mucilago spongiosa (Leysser) Morgan	F. A. Gilbert	18
Oligonema flavidum (Peck) Peck	Smart	52, 53
Perichaena corticalis (Batsch) Rost.		
as *Licea pannorum*	deBary	3
as *Licea pannorum*	Cienkowski	10
Perichaena depressa Libert	Constantineanu	11
	Smart	52, 53
Physarella oblonga (Berk. & Curt.) Morgan	Sinoto & Yuasa	49
	Smart	52, 53
Physarum albescens Macbr.		
as *P. fulvum*	Smith	54
Physarum bivalve Pers.		
as *P. sinuosum*	F. A. Gilbert	21
	Smart	52, 53
Physarum cinereum (Batsch) Pers.	F. A. Gilbert	21
	Smith	54, 55
	Smart	52, 53
Physarum compressum Alb. & Schw.	F. A. Gilbert	18, 21
	Kambly	33
Physarum crateriforme Petch	Abe	33
Physarum didermoides (Pers.) Rost.	Constantineanu	11
	Jahn	30
as *P. lividum* List.	Smith	54
	Smart	52, 53
	Kambly	34
Physarum digitatum G. Lister & Farquh.	Smart	52, 53
Physarum flavicomum Berk.	Smart	52, 53
Physarum globuliferum (Bull.) Pers.	Smart	52, 53
Physarum leucophaeum Fries	Smart	52, 53
Physarum leucopus Link		
as *Didymium leucopus*	Cienkowski	3
	Schunemann	48
	F. A. Gilbert	18, 21
	Smart	52, 53
Physarum notabile Macbr.		
as *P. connatum*	F. A. Gilbert	18, 21
	Smith	54
Physarum nucleatum Rex	Smart	52, 53
Physarum nutans Pers.		
as *P. albipes*	deBary	3
	Smart	52, 53
Physarum polycephalum Schw.	Howard	26
	Smart	52, 53
Physarum pulcherrimum Berk. & Rav.	Smart	52, 53
Physarum Serpula Morgan	F. A. Gilbert	18, 21
	Smith	54
	Smart	52, 53
Physarum straminipes Lister	Smith	55

Species	Worker	References
Physarum virescens Ditmar	F. A. Gilbert	18, 21
	Smith	54
Physarum viride (Bull.) Pers.	F. A. Gilbert	19, 21
	Smart	52, 53
Reticularia Lycoperdon Bull.		
as *R. umbrina*	deBary	3
as *R. umbrina*	McClatchie	43
	Jahn	29
	F. A. Gilbert	18, 19, 21
	Cook & Holt	12
	Wilson &	
	Cadman	59
	Smith	55
	Schunemann	48
	Smart	52, 53
	Kambly	33
Stemonitis axifera (Bull.) Macbr.		
as *S. ferruginea*	Jahn	29
as *S. ferruginea*	F. A. Gilbert	19, 21
as *S. ferruginea*	Smith	55
	Smart	52, 53
as *S. ferruginea*	Ellison	15
Stemonitis flavogenita Jahn	Smith	54, 55
Stemonitis fusca Roth.	Hoffmann	25
	deBary	3
	Constantineanu	11
	F. A. Gilbert	16, 19, 21
as *S. fusca* var. *rufescens*	F. A. Gilbert	21
	Smith	54
	Abe	33
	Smart	52, 53
	Ellison	15
Stemonitis splendens Rost.		
as *S. splendens* var. *flaccida*	Constantineanu	11
as *S. flaccida*	Jahn	28, 29
	F. A. Gilbert	19
as *S. splendens* var. *flaccida*	F. A. Gilbert	19, 21
as *S. splendens* var. *flaccida*	Cook & Holt	12
	Smith	54
as *S. splendens* var. *flaccida*	Sinoto & Yuasa	49
	Smart	52, 53
as *S. splendens* var. *flaccida*	Smart	52, 53
Trichia Botrytis (J. F. Gmel.) Pers.		
as *T. fragilis*	Lister	39
	Smith	54, 55
Trichia contorta (Ditm.) Rost.	F. A. Gilbert	21
	Smart	52, 53

Species	Worker	References
Trichia favoginea (Batsch) Pers.	Smith	54, 55
	Smart	52, 53
Trichia floriformis (Schw.) G. Lister	F. A. Gilbert	19, 21
as *T. lateritia*	Smith	55
	Smart	52, 53
Trichia persimilis Karst.	F. A. Gilbert	21
	Smith	54
	Smart	52, 53
Trichia pusilla (Hedw.) Martin		
as *T. fallax*	Lister	39
Trichia scabra Rost.	F. A. Gilbert	21
	Smith	55
Trichia varia (Pers.) Pers.	deBary	4
	F. A. Gilbert	21
	Cook & Holt	12
	Smart	52, 53
Tubifera ferruginosa (Batsch) J. F. Gmel.		
as *Tubulina cylindrica*	Jahn	30

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EXPLANATION OF FIGURES

Photomicrographs and Explanatory Drawings of Myxomycete Swarmcells. FIG. 1. *Ceratiomyxa fruticulosa.* FIG. 2. *Tubifera ferruginosa.* FIG. 3. *Cribraria purpurea.* FIG. 4. *Dictydium cancellatum.* FIG. 5. *Enteridium Rozeanum.* FIG. 6. *Arcyria cinerea.* FIG. 7. *Oligonema Schweinitzii.* FIG. 8. Zygote of *Oligonema Schweinitzii.* FIG. 9. *Trichia affinis.* FIG. 10. *Trichia favoginea.* FIG. 11. *Lamproderma arcyrionema.* FIG. 12. *Lamproderma Sauteri.* FIG. 13. *Lamproderma scintillans.* FIG. 14. *Stemonitis axifera.* FIG. 15. *Stemonitis fusca.* FIG. 16. *Stemonitis splendens.* FIG. 17. *Fuligo septica.* FIG. 18. *Leocarpus fragilis.* FIG. 19. *Physarella oblonga.* FIG. 20. *Physarella oblonga.* FIG. 21. *Physarum didermoides.* FIG. 22. *Physarum flavicomum.* FIG. 23. *Physarum melleum.* FIG. 24. *Physarum viride.* FIG. 25. *Didymium xanthopus.* All photographs and drawings are × 1000.

A NEW AQUATIC SPECIES OF PYTHIUM *

Adrian W. Poitras

(with 17 figures)

In the course of a study of the aquatic Phycomycetes of Illinois which has been in progress during the last several years, eighty-five isolates representing the genus *Pythium* have been obtained. It was found that these represented sixteen different species, including one whose characteristics are such that it is being proposed as new in the sub-genus Sphaerosporangium.

The described species so far recognized in Illinois are as follows: *Pythium monospermum* Pringsheim, *P. dissotocum* Drechsler, *P. gracile* Schenk, *P. tenue* Gobi, *P. catenulatum* Matthews, *P. torulosum* Coker and Patterson, *P. inflatum* Matthews, *P. aphanidermatum* (Edson) Fitzpatrick, *P. pulchrum* von Minden, *P. ultimum* Trow, *P. splendens* Braun, *P. vexans* de Bary, *P. complectans* Braun, *P. debaryanum* Hesse, *P. irregulare* Buisman.

The following new species was found:

Pythium multisporum sp. nov.**

Mycelio ramosiore, 3.0–8.4 μ diam. Sporangiis sphaericis vel subsphaericis, terminalibus; 29.5–55.0 μ diam.; proliferentibus; zoosporis 30–50, reniformibus, a latere biflagellatis, quae in vesica sporangio contigua formantur et sunt 8.4–10.5 μ lata, 10.5–16.8 μ longa si in cystibus gignuntur. Oogoniis sphaericis, subsphaericis vel enormibus; levibus; terminalibus vel subterminalibus, quae si singula gignuntur sunt 18.9–39.9 μ diam., si composita, 26.4–48.8 μ lata, 39.6–74.8 μ longa. Oosporis sphaericis vel subsphaericis; 10.5–29.4 μ diam.; apleroticis; quae singulae vel plures singulis oogoniis insunt et cuticulas leves et paulo crassiores habent. Antheridiis androgynis et diclinis, quae singula vel plura singulis oogoniis insunt et quorum pars extrema ad oogonium adhaeret.

** Latin diagnosis prepared with the assistance of Prof. R. P. Oliver, Department of Classics, University of Illinois.
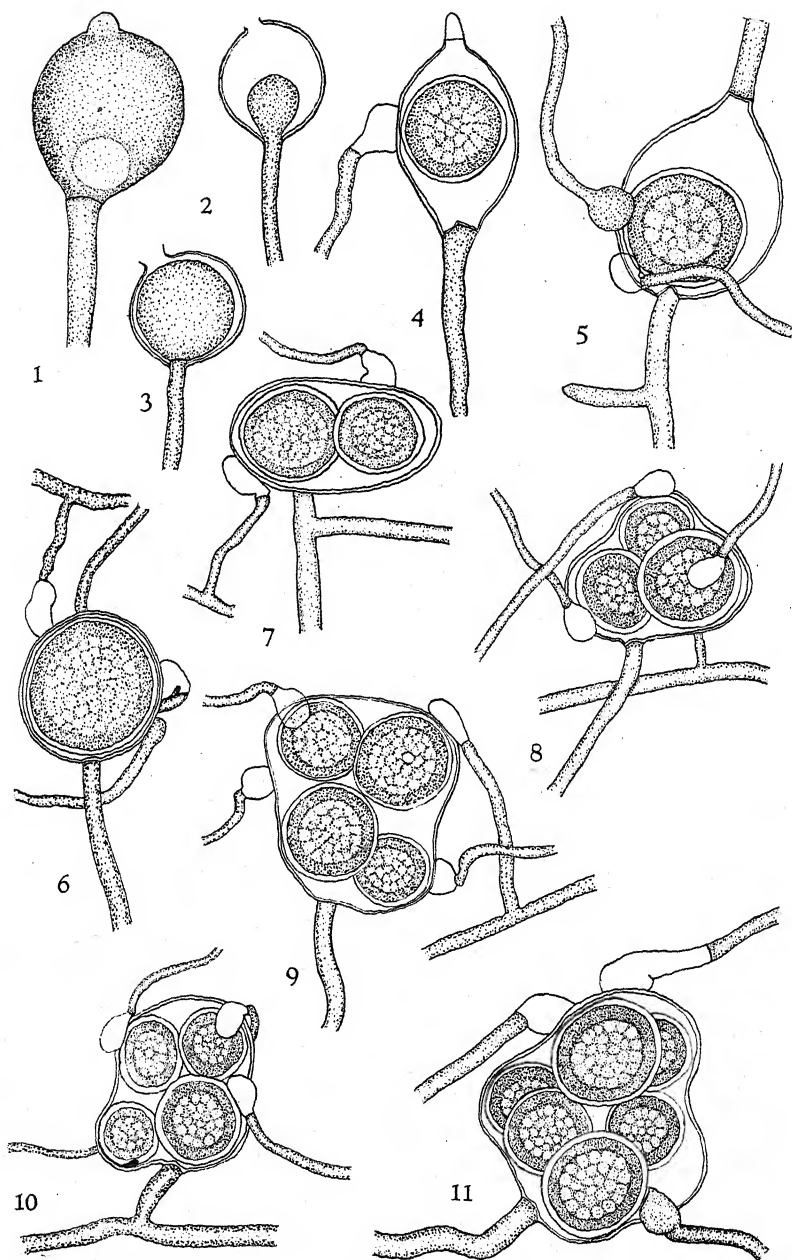
FIGS. 1–11. *Pythium multisporum.*

Hyphae freely branched and well developed on most culture media, measuring 3.0–8.4 μ in diameter.   Sporangia spherical, subspherical, infrequently ellipsoidal or pyriform, occasionally papillate; terminal on lateral branches; measuring 29.5–55.0 μ, average 42.8 μ in diameter; proliferous, the secondary sporangia usually formed within the walls of the primary ones; zoospores 30–50, laterally biflagellate and reniform, formed within a vesicle borne adjacent to the sporangium and measuring 8.4–10.5 $\mu \times$ 10.5–16.8 μ upon encystment.   Oogonia spherical, ovoid or irregular, smooth; terminal or intercalary, unisporous oogonia measuring 18.9–39.9 μ, average 30.4 μ in diameter, multisporous oogonia measuring 26.4–48.4 $\mu \times$ 39.6–74.8 μ, size largely dependent upon the number of oospores.   Oospores spherical to ovoid, one to several per oogonium, aplerotic, measuring 10.5–29.4 μ, averaging 21.6 μ in diameter, with a smooth, moderately thickened wall.   Antheridia androgynous and diclinous, one to several, usually two, per oogonium; the antheridial stalk of variable length; the antheridial cell cut off by a cross-wall, clavate to swollen, closely to moderately applied terminally to the oogonium, measuring 6 to 10 μ by 9 to 15 μ.

Isolated from a soil sample taken from the bank of a branch of the Illinois River at Havana, Illinois, on October 19, 1947.

This fungus grows quite readily on most types of agar media and on boiled hempseed in water.   Growth on agar media is limited usually to surface and subsurface mycelia; however, scant aerial hyphae developed on malt agar.   The mycelial growth on malt, malt-peptone and potato-dextrose agar media is quite profuse, whereas on corn-meal agar growth is very sparse.   All stages are produced readily on hempseed in water and, therefore, most of the material used for this study was grown by this method.

The most distinguishing characteristic of this fungus is that many of its oogonia contain more than one oospore. While about seventy-seven per cent typically contain a single oospore, about seventeen per cent contain two oospores, five per cent contain three oospores and the remaining one per cent contain from four to six oospores. Sorokin (1872) described *Pythium polysporum* as a species in which the oogonia contained several oospores, the oogonia measuring 40.0–50.0 μ in diameter and the oospores measuring 7–8 μ in diameter. Butler (1907) excludes *P. polysporum* from the genus *Pythium* on the basis of its sexual and asexual stages,

and both Matthews (1931) and Middleton (1943) concur in this opinion, Matthews suggesting that it might represent a new undiagnosed genus of the Pythiaceae. It is quite evident that our isolate is not the same as the one described by Sorokin.

Oogonia with two oospores are infrequently produced in a limited number of species of *Pythium*. The most closely related of these to *Pythium multisporum* is *Pythium pulchrum*, but *P. pulchrum* differs from our isolate by possessing the following characteristics: catenulate sporangia, the presence of a discharge tube of variable length between the sporangium proper and the vesicle, the lack of internal sporangial proliferation, and hypogynous antheridia.

Internally proliferating sporangia have been reported for several species of *Pythium*, including *P. proliferum, P. marsipium, P. polytylum, P. helicoides, P. oedochilum, P. palingenes,* and *P. nagii*, but none typically produce more than one oospore in an oogonium. *Pythium proliferum* differs also from *P. multisporum* in that its sporangia and oogonia are smaller in diameter and that hypogynous antheridia are characteristic. *Pythium marsipium* has large spherical or asymmetrically utriform sporangia which are papillate while *P. polytylum* has several reserve globules within the oospores and elongate, cylindrical or irregularly shaped antheridia. *Pythium helicoides* is distinguished from our isolate also by the fact that the antheridial stalk is coiled around the oogonial stalk. Papillate sporangia and antheridia which are wavy, contoured and attached over their entire length serve to separate *P. oedochilum* and *P. palingenes* from *P. multisporum*. *Pythium nagii* is obviously distinguished from our isolate because of the small size of its sexual and asexual structures, the proliferous nature of its sporangia being the only factor serving to unite it with this group. The presence of only a moderately thickened wall and a single reserve globule in the oospore, the fact that oogonia characteristically often possess several oospores, and the fact that the sporangia are regularly spherical to subspherical serve adequately enough to separate *P. multisporum* from other described species.

The pathogenicity of *Pythium multisporum* has not been studied.
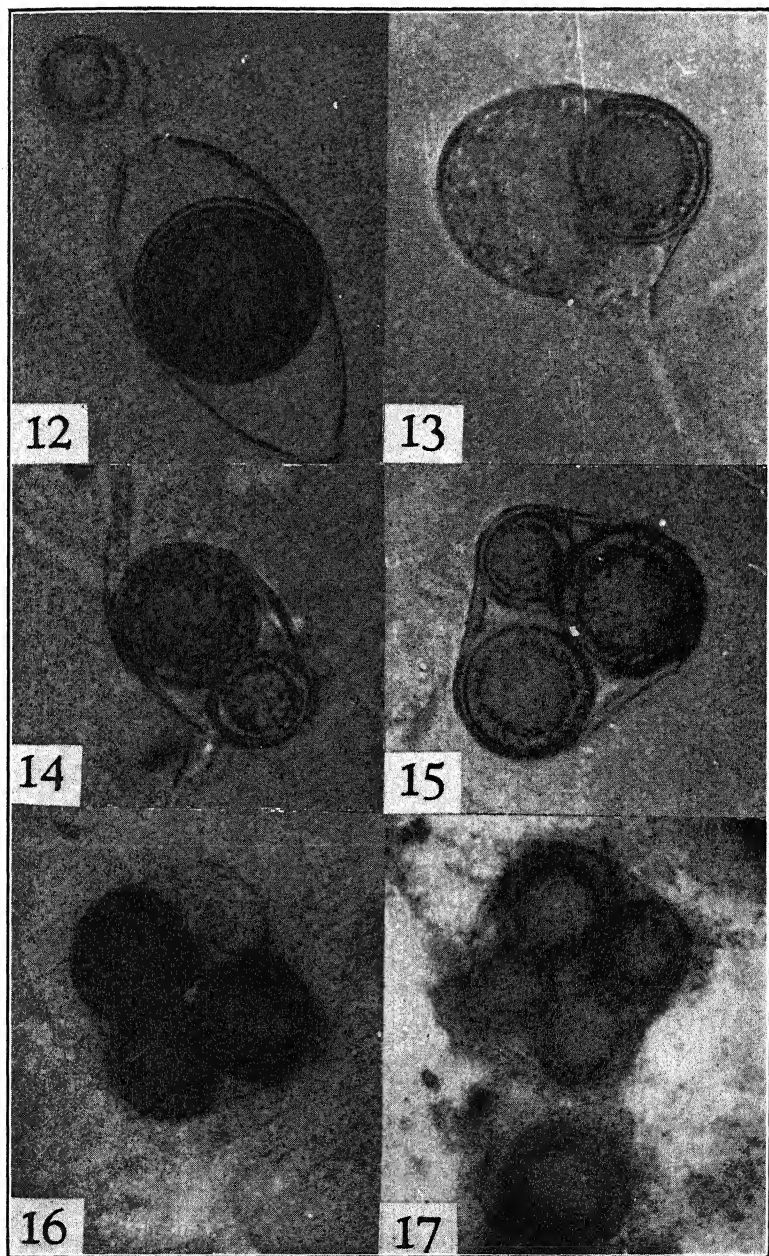
FIGS. 12–17. *Pythium multisporum.*

SUMMARY

From soil and water samples taken throughout the State of Illinois during the past several years sixteen species of the genus *Pythium* have been isolated and identified, including a new species belonging to the sub-genus Sphaerosporangium here described and named whose most destinctive characteristic is that, for a species of *Pythium,* a relatively high per cent of the oogonia possess from two to six oospores.

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EXPLANATION OF FIGURES

FIGS. 1, 2, and 3: terminal sporangia, figures 2 and 3 showing proliferation. FIGS. 5, 6, 7, 8, 9, 10 and 11 of oogonia; figures 4, 5, and 6 showing variation in terminal and intercalary single spored oogonia; figure 7, an oogonium with two oospores; figure 8, an oogonium with three oospores; figures 9 and 10, oogonia with four oospores; figure 11, an oogonium with six oospores. (All figures magnified 600 ×.)

FIGS. 12–17. Photomicrographs of oogonia. Figure 12, chain of two oogonia representing extremes in oospore size; figures 13 and 14, oogonia with two oospores, the oospore on the left in figure 13 being aborted; figure 15, an oogonium with three oospores, each oospore being of different size; figures 16 and 17 are oogonia with four oospores, one oospore in figure 16 being aborted. (Figure 12 approximately 800 ×, others 575 ×.)

PRESERVATION OF SAPROLEGNIACEAE BY THE MINERAL OIL METHOD

Helen Simpson Reischer [1]

The mineral oil method, originally developed for the conservation of bacterial cultures, has been applied to filamentous fungi first by Sherf (1943), later by Norris (1944) and Wernham (1946), and most widely by Buell and Weston (1947). The latter remarked upon the peculiar applicability of this simple method, which they used successfully in preserving a collection of 1800 cultures including, as well as higher fungi, Actinomycetes and terrestrial Phycomycetes, to delicate non-sporulating forms.

At the time when Dr. Weston was carrying on this work the author was faced with the problem of maintaining in pure culture a number of aquatic Phycomycetes of the family Saprolegniaceae. These fungi were being kept on Difco corn-meal agar slants in a 4–10 degree Centigrade coldroom. Such slants have a very uncertain life, drying more or less unpredictably in from three to fifteen or nineteen months. Many species of this family produce resistant spores (oospores), for which a successful method of germination has recently been reported (Ziegler, 1948). These spores have been reported as requiring a resting period, the limits of which are not precisely known, so that cultures derived from oospores would not always be readily available. Oospores do not appear suited to lyophilization. Of twelve isolates of *Achlya* with abundant mature oospores which were lyophilized, using the method of Raper and Alexander (1945), none were viable three months later. Microscopic examination revealed disorganization of the oospores. The encysted zoospores (cystospores) of seven isolates of *Achlya* disintegrated under lyophilization. The oospore-producing species then, as well as the various forms of heterothallic

species and those homothallic species which typically produce few if any mature oospores, must be considered for purposes of maintenance as non-sporulating mycelia for which the mineral oil method might be suitable.

As they were obtained, seventy-one isolates, including eight genera, were grown on Difco corn-meal agar slants which were then flooded with mineral oil, following the method of Buell and Weston (1947), and subsequently incubated at 4–10 degrees Centigrade. Of these, four isolates (*Achlya* and *Brevilegnia*) were lost in 1946 at 6–11 months. At the same time 11 slants of other isolates (*Achlya*) were discarded—four because they failed

TABLE 1

RETENTION OF VIABILITY BY CULTURES OF SAPROLEGNIACEAE
UNDER MINERAL OIL

Genus	Number of different isolates	Age of oldest culture or cultures viable May, 1948
Achlya	5	30 months
	1	24 months
	5	19 months
	1	17 months
	1	14 months
	3	13 months
	4	12 months
	4	11 months
Dictyuchus	1	24 months
	1	17 months
Isoachlya	3	15 months
	4	13 months
Leptolegnia	1	30 months
	1	12 months
Pythiopsis	2	15 months
	5	13 months
	1	12 months
Saprolegnia	1	30 months
	6	24 months
	4	13 months
	4	12 months
	4	10 months
Thraustotheca	1	12 months
	4	11 months

to grow on being subcultured, seven because their revival was long delayed, and assumed or observed to be the result of the germination of oospores: other slants of the same isolates have now, however, retained viability under oil for longer periods. It is suggested that a possible reason for these early failures lies in the use of unsupplemented corn-meal agar, on which *Achlya* and *Brevilegnia* in particular showed relatively slow growth. In May, 1948, subcultures were made of the 133 slants of the remaining sixty-seven isolates on corn-meal agar (forty-four isolates were maintained as single slants, twenty-three isolates as 2–6 slants each) which had been under mineral oil for varying periods, with the results shown in table 1. All were viable.

These results show that the mineral oil method can be successfully applied to the preservation of these delicate fungi.

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CONIDIA–LIKE STRUCTURES IN PLEC–TANIA COCCINEA

Const. J. Alexopoulos and Ed. E. Butler

(with 1 figure)

On May 3, 1947 several ascocarps of *Plectania coccinea* (Scop.) Fuckel were collected in Sanford woodlot, East Lansing, Michigan. Upon microscopic examination of ascospores scraped off the hymenial layer, it was found that a number of them were already germinating by means of one to four germ tubes each of which cut off a conidium-like cell at the tip. The germ tubes varied in length from approximately half the length of the ascospore to three times that length. A number of detached "conidia" were also found in the same mount.

An attempt was made to induce this phenomenon by germinating ascospores on agar and in water. On May 5, ascospores were caught on the surface of potato dextrose agar in a Petri dish inverted over a puffing apothecium which had been kept in a moist chamber for two days. Several ascospores germinated, but no "conidia" were formed. Spores from a puffing apothecium caught on a dry glass slide subsequently placed in a moist chamber failed to germinate.

An apothecium collected on May 13 was placed in a moist chamber. On May 15 ascospores were caught on a glass slide and mounted under a cover glass in distilled water. The slide, supported on two pieces of glass rodding, was placed in a Petri dish into which some water was poured. In 24 hours numerous ascospores situated near the edge of the cover glass had germinated by means of one to four germ tubes. The percentage of germinated ascospores diminished in proportion to their distance from the edge of the cover glass. No conidia-like bodies had been formed as yet, but typical constrictions in the germ tubes some distance from the tip suggested that they were in the process of formation. Indeed on May 17, 45 hours after ascospore ejection from the asci, numerous "conidia" were observed completely formed at the tips of the germ tubes.
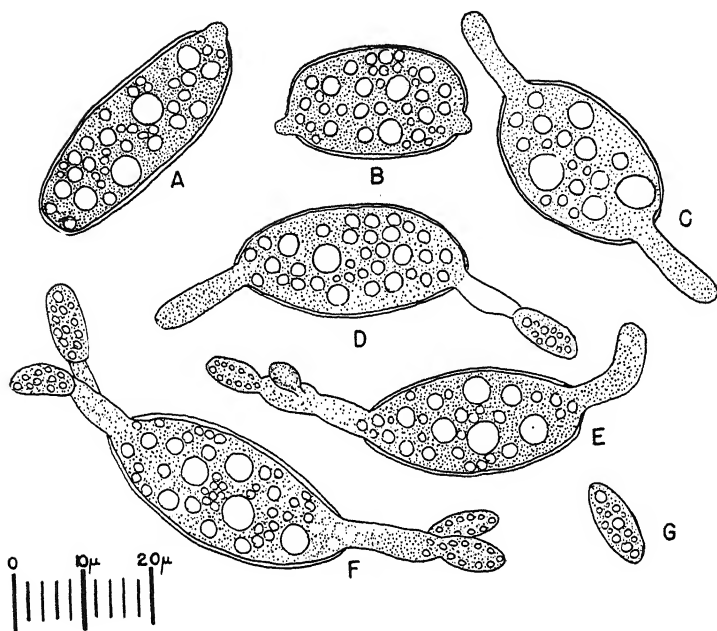
Fig. 1. *Plectania coccinea*. (A–C) Germinating ascospores. (D–F) Formation of conidia-like structures. (G) Mature "conidium."

Collections from three localities near East Lansing were made and observations were repeated in the spring of 1948 when the ascocarps of *Plectania coccinea* again made their appearance. Scrapings off the surface of the hymenium always yielded ascospores with germ tubes and "conidia" (FIG. 1). If the apothecia were permitted to remain in a moist chamber for a few days, the number of germinated ascospores and especially of detached "conidia" increased greatly. "Conidia" were invariably produced by the time the germ tube had grown to half the length of the ascospore. In some cases "conidia" were found budding directly from the ascospore.

One apothecium was left in a moist chamber for a period of two weeks. Scrapings off the hymenial layer were mounted in water at the end of that period. The hymenium was in a state of advanced disintegration, but a very large number of the conidia-like cells were evident among various contaminating organisms. All ascospores had completely disintegrated.

The conidia-like structures produced by *Plectania coccinea* resemble the ascospores of this fungus closely except for their size. The average size of the ascospores in the material at hand was found to be 14.5×31.5 μ while the average size of the "conidia" was 4.5×11 μ. The range in size of the "conidia" was 3.7–5×9–13 μ.

The exact nature of the conidia-like cells abundantly produced by *Plectania coccinea,* as reported above, is not clear. Germination has not been observed in any of the preparations in which these cells were found, but no extensive germination studies were undertaken.

Similar structures arising in the same way from germinating ascospores of *Peziza vesiculosa* and *Bulgaria inquinans* were observed in 1865 by the Tulasne brothers (3). Copious "conidiola" were observed on short, thick germ tubes arising from the ascospores. Brefeld (2) records production of similar structures he calls conidia, from germinating ascospores of *Coryne sarcoides,* but not from *Plectania coccinea* of which fungus he says, ". . . brachte nur unfruchtbare weisse, üppige Mycelien." He does not describe the germination of the ascospores in the latter species.

Budding of ascospores of several species of ascomycetes is a well known phenomenon. In the Taphrinales and yeasts budding is of common occurrence. Bond (1), who describes the production of sprout cells from the ascospores of *Sphaerulina mappiae,* gives a brief review of the literature on budding ascospores.

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CREBROTHECIUM ASHBYI [1]

JOHN B. ROUTIEN [2]

During the course of a review of the literature on *Spermoph-thora, Eremothecium, Ashbya* and *Nematospora* the writer became aware of the fact that though the first species described for the genus *Eremothecium, E. Cymbalariae* Borzi, had been validly published (2), another name, *E. Ashbyi,* had been proposed first in 1935 (3) for a second fungus but had not been published validly according to strict interpretation of Article 38 of the International Rules of Botanical Nomenclature (5) in that the description was published after January 1, 1935 and, unfortunately, was not accompanied by a Latin diagnosis. Though many mycologists would disregard such a point, it seems to the writer that the description of the fungus in question should be published effectively and validly without more delay.

Guilliermond (3, 4) considered that the fungus he was describing best fitted into the genus *Eremothecium.* There were many similarities between his fungus and *E. Cymbalariae,* it is true: The manner of growth was the same in each; asci (or sporangia ?) arose in each in a similar manner from multinucleate cells; the spores had the same shape. However, there were several ways in which the new fungus differed from *E. Cymbalariae:* in *Eremothecium* the asci were described as solitary at the apices of hyphae, lageniform or nearly so, with 30 or more spores in each, and the spores were arranged in two clusters at opposite ends of the ascus; in the new fungus, on the other hand, the asci usually were arranged in a series of several in an intercalary position and only occasionally solitary, they were ellipsoidal-truncate, there were 4–32, mostly 6–18 and rarely fewer than 4 or more than 32, spores in

each sporogenous cell, and though the spores sometimes showed a vague clustering they were always entangled and overlapped one another.

In some ways it might be best merely to validate the combination *Eremothecium Ashbyi,* but the writer believes that the differences enumerated in the previous paragraph are so great that the two fungi should be placed in different genera.

The best classification of these two fungi seems to the author to be to regard them as non-sexual forms derived from a *Spermoph-thora*-like organism and to place all three in the same family. Guilliermond (4) reported that a culture of *S. Gossypii* after 8 years of cultivation had ceased to reproduce sexually but had a form of asexual reproduction which basically was the same as that demonstrated by the two organisms here discussed. Moreover, Ashby and Nowell (1) had stated earlier that in *S. Gossypii* "The spores germinate by germ-tubes which (1) develop into the primary mycelium or (2) fuse in pairs. . . ." It seems quite possible, then, that forms like *Eremothecium* could easily be derived from a form like *Spermophthora* by a disappearance of sexual reproduction.

The name *Crebrothecium* is derived from *creber* (frequent, numerous or repeated), in reference to the numerous sporogenous cells, and *theca.*

Crebrothecium gen. nov.

Hyphis dichotome ramosis; cellulis fertilis truncato-ellipsoideis, saepius catenatis sed interdum singularibus; pariete asci deliquescente; ascosporis saepius 8–16 in asco quoque, sed interdum paucis vel multis, irregulatim dispersis vel in fasciculis indefinitivis, clavato-acicularis, rectis vel saepe curvulis, continuis, una parte sporae vacua et fastigata; ex sporis germinantibus oritur mycelium primigenium.

Hyphae branched dichotomously; sporogenous cells truncate-ellipsoidal, usually in chains but occasionally solitary; wall of ascus becoming deliquescent; spores mostly 8–16 in each ascus, but occasionally few or more, irregularly disposed or sometimes in vague clusters, clavate-acicular, straight or often curved, simple, a part of the spore devoid of contents and attenuated to a pointed prolongation; spores germinating to produce primary mycelium.

The type species is *Crebrothecium Ashbyi* (Guill.) Routien.

C. Ashbyi (Guill.) comb. nov.

Mycelio postremo lutescente; hyphis 2.7–5.5 μ latis; ascis 68–87 × 14–16 μ; sporis 29–31.8 × 2–2.8 μ. Parasito in capsulis *Gossypii* in Sudan.

Mycelium becoming yellow; hyphae 2.7–5.5 μ wide; sporogenous cells 68–87 × 14–16 μ; spores 29–31.8 × 2–2.8 μ. Parasitic in capsules of *Gossypium* in Sudan.

Type: Figures 1, 6, 7, 13, 18, 19 in Guilliermond (4).

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THE INFLUENCE OF CONCENTRATIONS OF NUTRIENTS, THIAMIN, AND BIOTIN UPON GROWTH, AND FORMATION OF PERITHECIA AND ASCO–SPORES BY CHAETOMIUM CONVOLUTUM [1]

Virgil Greene Lilly and H. L. Barnett

(with 4 figures)

An adequate supply of a given vitamin has been found to be essential for fruiting by some vitamin-deficient fungi. *Sordaria fimicola* (1, 7) was found to require more biotin for the formation of perithecia and ascospores than for vegetative growth. The amount of biotin required for the formation of ascospores was greater than that required for the formation of perithecia. The absolute amount of biotin required for the formation of perithecia decreased as the amount of nutrients in the medium decreased. Similar results have been reported for *Ceratostomella fimbriata* (2) which is deficient for thiamin. The amount of thiamin required by this fungus for the production of perithecia decreased as the amount of nutrients in the medium decreased. The numbers of perithecia produced by these two fungi depended upon the amount of nutrients present as well as upon the concentrations of the vitamins employed. Hawker (3) found that the concentration of glucose optimal for the fruiting of sixteen fungi increased as the concentration of growth substances increased.

It was considered desirable to extend the study of the interrelations among nutrient supply, vitamin concentrations and sexual reproduction to another fungus. *Chaetomium convolutum* Chivers was found to be deficient for both thiamin and biotin. This paper reports the interrelations between nutrient supply and concentrations of thiamin and biotin as they affect the growth and sexual reproduction of this fungus.

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 397.

MATERIALS AND METHODS

The isolate of *C. convolutum* used was obtained from a contaminated plate. Preliminary study showed that this isolate fruited readily under certain laboratory conditions, and was deficient for thiamin and biotin.

The basal liquid medium used had the following composition:

Glucose.............................. 25 gm.
KH_2PO_4............................. 1 gm.
$MgSO_4.7H_2O$......................... 0.5 gm.
Fumaric acid.......................... 1.32 gm.
Casein hydrolysate equivalent to.......... 2 gm. casein
Na_2CO_3............................. 1.1 gm.
Fe+++............................. 0.2 mg.
Zn++............................. 0.2 mg.
Mn++............................. 0.1 mg.
Double-distilled water to make...........1000 ml.

Leonian and Lilly (6) described a method of preparing this medium in essentially vitamin-free condition. The pH of the medium was adjusted to 6.5 before autoclaving by adding sodium hydroxide or hydrochloric acid. The pH of the medium was approximately 6.0 after autoclaving. Thiamin and biotin were added to the medium before autoclaving in the amounts specified in connection with the experiments. The concentrations of the vitamins are given in micrograms (μg.) per flask.

The culture vessels used were 250 ml. pyrex Erlenmeyer flasks which had been cleansed in dichromate-sulfuric acid solution. Twenty-five ml. of medium were used per flask. Inoculation was made by adding a loopful of spore suspension to each flask. The temperature of incubation was $25 \pm 1°$ C.; the time of incubation varied and is reported in connection with the experiments.

The number of flasks prepared for each concentration studied varied from four to ten, depending upon the experiment. The amount of growth was followed by harvesting duplicate flasks and weighing the mycelium after it had been dried to constant weight. The weights are reported in milligrams, each datum being the average of two single determinations. The pH of the culture medium was determined at time of harvest by means of glass electrode assembly. Perithecia were considered present when they could be

seen as small black dots with the unaided eye. The numbers of perithecia were estimated by rough counts and the degree of maturity was determined by microscopic examination. The presence of ascospores was detected by crushing perithecia under a cover slip on a slide.

EXPERIMENTAL RESULTS

Effect of biotin and thiamin upon growth and formation of perithecia: Two extensive experiments were made to determine the effect of varying concentrations of thiamin and biotin upon growth, the pH of the culture medium, and the formation of perithecia. Since exogenous thiamin and biotin are required by this fungus, it was necessary to keep the concentration of one vitamin constant while the concentration of the other varied. A portion of the data will be presented in the form of graphs.

In the first experiment, the concentration of thiamin was kept constant (2.5 μg. per flask) while the concentration of biotin varied
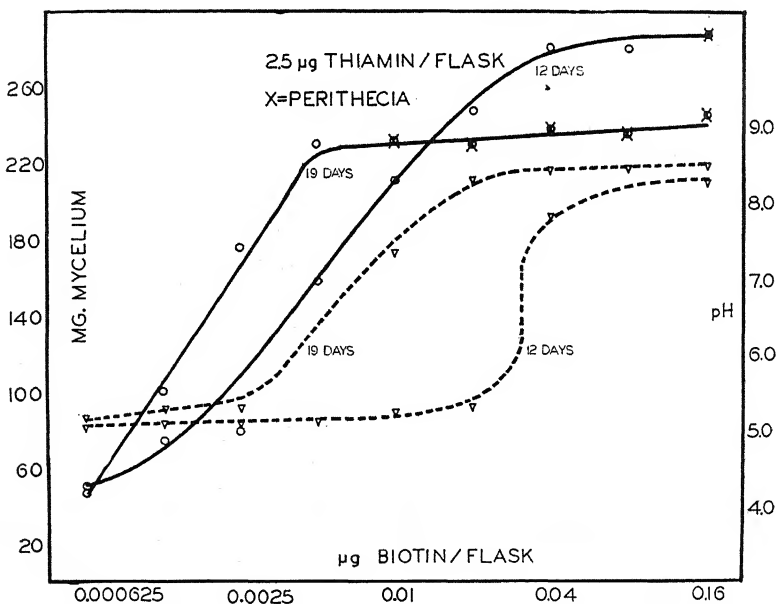
Fig. 1. Effect of varying concentrations of biotin on growth, pH of the culture medium and formation of perithecia at 12 and 19 days of incubation. Undiluted medium. ————, growth; ———————, pH; X, perithecia.
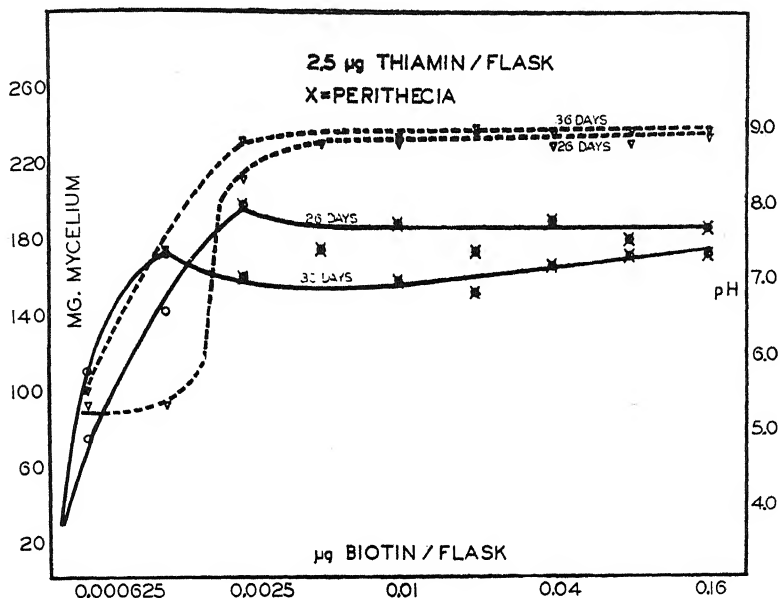
FIG. 2. Effect of varying concentrations of biotin on growth, pH of the culture medium and formation of perithecia at 26 and 36 days of incubation. Undiluted medium. ————, growth; - - - - - - - -, pH; X, perithecia.

from 0.0 to 0.16 μg. per flask.   Figures 1 and 2 show that growth was proportional to the amount of biotin in the medium when the concentration of biotin was less than 0.04 μg.   The time of incubation required to produce the maximum weight of mycelium with the lower concentrations of biotin increased as the concentration of biotin decreased.   Autolysis followed soon after maximum weight was attained.   The pH of the culture medium remained near 5 during early growth; as the weight of mycelium approached a maximum, the pH increased to 8–9.   Presumably this increase in pH is connected with the process of autolysis.

The concentration of biotin influenced the time of incubation required for perithecia to form, and to some extent the numbers produced.   While a concentration of biotin greater than 0.04 μg. had but little effect upon growth, these higher concentrations were more effective in the rapid production of perithecia.   Thus perithecia were produced by the twelfth day when the concentration of biotin was 0.16 μg., whereas fifteen days were required when the concen-

tration was 0.08 and 0.04 μg. The time of appearance of peri-
thecia in the same series of flasks varied somewhat; the time of
perithecial formation recorded in figures 1 and 2 is that found when
half or more of the flasks in a given experiment exhibited peri-
thecia visible to the unaided eye. This variation in time of peri-
thecial formation was seldom greater than one day when the con-
centrations of biotin were greater than 0.04 μg. As the concentra-
tion of biotin was decreased further, this variation increased to as
much as a week (lowest concentrations). The pH of the culture
medium was always greater than 7 when perithecia formed. How-
ever, the flasks receiving 0.00625 μg. of biotin did not produce peri-
thecia after 49 days of incubation and the pH was 7.57 at this time.

The effects of varying concentrations of thiamin in the presence
of 0.16 μg. biotin upon growth, the pH of the culture medium, and
on perithecial formation are presented in figures 3 and 4. The
amount of growth was proportional to the concentration of thiamin
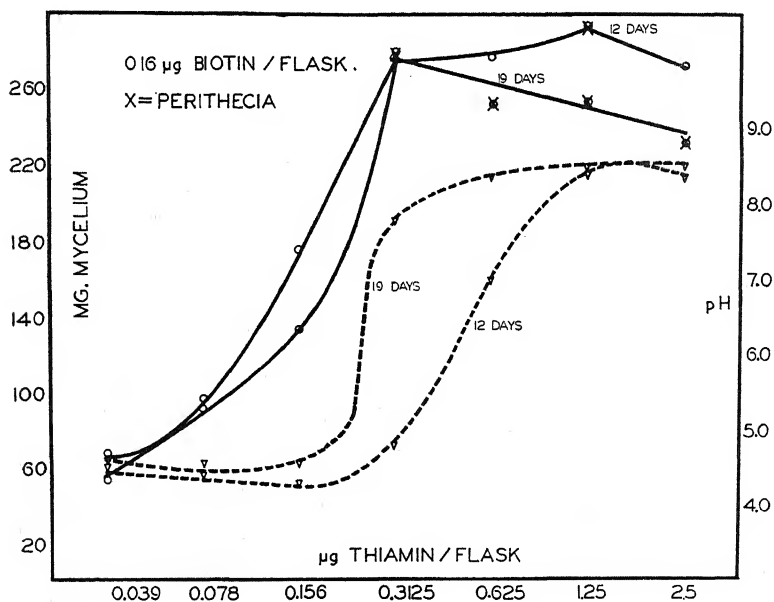
FIG. 3.   Effect of varying concentrations of thiamin on growth, pH of the
culture medium and formation of perithecia at 12 and 19 days of incubation.
Undiluted medium. —————, growth; --------, pH; X, perithecia.
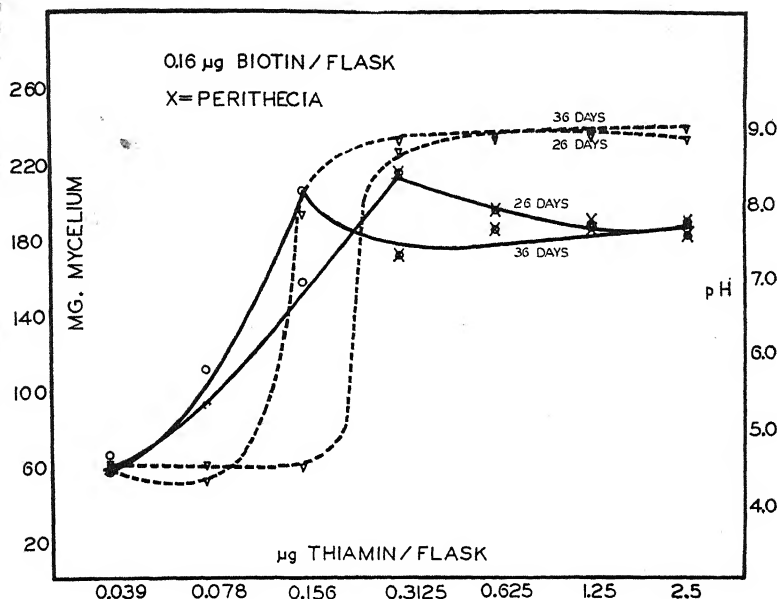
FIG. 4. Effect of varying concentrations of thiamin on growth, pH of the culture medium and formation of perithecia at 26 and 36 days of incubation. Undiluted medium. ————, growth; --------, pH; X, perithecia.

in the medium in the range below 0.3125 μg. Concentration of thiamin greater than this had but little effect on the amount of mycelium produced. However, the formation of perithecia was induced sooner in the higher concentrations of thiamin. While the changes in the pH of the culture medium resembled those in the biotin-series, it may be noted that in the lower thiamin concentrations the pH fell almost one unit below that of the biotin series. In general, the change in pH to the alkaline range was accompanied by a rapid increase in the rate of growth.

Dilution of the medium and the absolute amounts of the vitamins required for the formation of perithecia and ascospores: The experiments described above employed full strength media. The results showed that, for a given period of incubation, there were critical concentrations of thiamin and biotin below which perithecia did not form. The following experiments were made to determine if the amount of nutrients in the medium affected these criti-

cal concentrations. Full strength medium and one-fourth and one-sixteenth dilutions were used. In order to lessen the number of flasks required, only the lower concentrations of thiamin and biotin were used. Harvests were made at the time when perithecia were first observed. Some of the flasks were reserved so that microscopic examination could be made of the perithecia and ascospores. The results of this examination are given in tables 1 and 2.

TABLE 1

THE EFFECTS OF VARYING CONCENTRATIONS OF BIOTIN AND NUTRIENTS UPON GROWTH, pH OF THE CULTURE MEDIUM, AND THE NUMBERS AND DEGREES OF MATURITY OF PERITHECIA FORMED.

2.5 μg. thiamin hydrochloride per flask

μg. biotin/ flask	mg. mycelium	pH at harvest	Days incubation to form perithecia	Total number of perithecia formed and per cent mature
Undiluted Medium				
0.0	1	6.0	None at 60 days	None
0.0025	161	7.67	46	12,000, none mature, no ascospores
0.005	204	8.66	28	10,000, few mature, only an occasional ascospore
0.01	194	8.78	28	15,000, 25% mature
$\frac{1}{4}$ Dilution				
0.0	1	6.45	None at 60 days	None
0.000625	55	7.56	30	2000, 65% mature
0.0025	68	7.80	20	4000, 80% mature
0.005	67	7.61	15	5000, 85% mature
$\frac{1}{16}$ Dilution				
0.0	1	6.45	None at 60 days	None
0.0003125	15	7.23	17	500–700, less than 25% mature
0.000625	16	7.90	15	500–700, 50% mature
0.00125	21	7.94	12	500–700, 65% mature

It may be seen from tables 1 and 2 that the formation of perithecia by *C. convolutum* is dependent upon at least three factors: the concentration of thiamin, biotin, and of nutrients. For each concentration of nutrients there is a fixed critical concentration of thiamin and biotin below which perithecia do not form. A given

TABLE 2

The Effects of Varying Concentrations of Thiamin Hydrochloride and Nutrients Upon Growth, pH of the Culture Medium, and the Numbers and Degree of Maturity of Perithecia Formed.

0.16 μg. biotin per flask

μg. thiamin/ flask	mg. mycelium	pH at harvest	Days incubation to form perithecia	Total number of perithecia formed and per cent mature
Undiluted Medium				
0.0	1	5.05	None at 60 days	None
0.078	171	4.92	None at 60 days	None
0.156	149	8.41	54	10,000, none mature, no asco-spores
0.3125	220	8.00	24	10,000, few mature, few asco-spores
0.625	220	7.96	17	13,000, 25% mature
$\frac{1}{4}$ Dilution				
0.0	3	5.55	None at 60 days	None
0.039	44	7.96	60	1500–3000, 80% mature
0.078	58	8.50	28	2100–3000, 80–90% mature
0.156	75	8.12	12	3000, 90% mature
$\frac{1}{16}$ Dilution				
0.0	1	5.10	None at 60 days	None
0.0195	17	7.81	15	400–500, 50% mature
0.039	22	7.94	12	400–500, 50% mature

concentration of the vitamins may be above or below the critical concentrations, depending upon the concentration of nutrients in the medium. In general, the time of incubation required to produce perithecia in the presence of fixed amounts of the vitamins decreases as the medium becomes more dilute. Thus, the days required for perithecia to form when the biotin concentration was 0.025 μg. decreased from 46 to 20 and 12 days, respectively, when the medium was diluted to one-fourth and one-sixteenth full strength. However, perithecia did not form under any condition tried in less than ten days. The formation of perithecia appears to be connected with exhaustion of available nutrients.

A microscopic examination of the perithecia formed under the conditions of the previous experiment was made on the sixtieth day of incubation. At that time, the numbers of perithecia were

estimated by rough counts, and the maturity of the perithecia determined by examination. A perithecium was judged mature if it contained ascospores (the asci had degenerated in most instances by the time the examination was made). The relative number of mature and immature perithecia were estimated by rough counts under the microscope. The relevant data are given in tables 1 and 2.

DISCUSSION

In the presence of a given amount of nutrients (thiamin and biotin present) vegetative growth takes place first. The amount of vegetative growth is controlled by the amount of nutrients present and by the concentrations of thiamin and biotin. Only as vegetative growth attains a maximum do perithecia form. When the concentration of nutrients is the factor limiting growth, perithecia form only after the attainment of maximum growth. For any concentration of nutrients certain concentrations of thiamin and biotin are required before mature perithecia develop. Lower concentrations of these two vitamins allow perithecia to form, but these may never mature and form ascospores. At still lower concentrations of these vitamins perithecia do not form at all.

As the amount of nutrients in the medium is reduced lower concentrations of thiamin and biotin are required for sexual reproduction. Under these conditions the amount of vegetative growth is reduced and it may be deduced that each unit weight of mycelium must contain certain concentrations of these vitamins before sexual reproduction is possible. Therefore, the *relative* amounts of nutrients and thiamin and biotin determine whether perithecia and ascospores form and also control the time of incubation required for their production.

The general conclusions reached in our studies on the factors controlling sexual reproduction of *Sordaria fimicola* and *Ceratostomella fimbriata* were confirmed in this study of *Chaetomium convolutum*. More work will be required to determine the general validity of these conclusions with regard to the sexual reproduction of other vitamin-deficient fungi. It is to be expected that other nutritional factors may prove to be of equal importance as other fungi are studied.

Hawker (4) has found nutritional factors in addition to the vitamins to be of great importance for the fruiting of *Melanospora destruens*. Not only the concentration of the sugar, but the kind used in the medium, was of great importance for the production of perithecia by this species. Thus, 50 grams of sucrose per liter allowed the formation of numerous perithecia; none were formed under comparable conditions when the same weight of glucose was used. A few perithecia formed when the amount of glucose was reduced to 5 grams per liter. On the other hand, glucose supported greater vegetative growth of *M. destruens* than sucrose.

Klebs (5) fully recognized the role of specific constituents as well as concentration of the medium in reproduction by the fungi. His generalization that reproduction takes place under more restricted environmental conditions than growth is confirmed in this paper.

SUMMARY

Chaetomium convolutum is deficient for both thiamin and biotin. In the presence of an optimum amount of one of these vitamins growth was proportional to the amount of the other vitamin in the medium. The formation of perithecia and ascospores followed the attainment of maximum vegetative growth provided that the concentrations of these vitamins exceeded certain values. The absolute amounts of thiamin and biotin required for the formation of perithecia and ascospores were a function of the concentration of the nutrients in the medium. The numbers of perithecia formed for a given concentration of nutrients increased somewhat as the concentrations of the vitamins were increased, the numbers of mature perithecia formed were more nearly proportional to the concentrations of the vitamins. This was also true of the numbers of ascospores formed. The amounts of thiamin and biotin required for vegetative growth, perithecial formation and the production of ascospores in the presence of a given amount of nutrients increased in the above order.

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DOWNY MILDEW OF URTICA IN THE UNITED STATES [1]

CHARLES GARDNER SHAW [2]

(WITH 1 FIGURE)

Berkeley (2) in 1846 was the first to mention the occurrence of a downy mildew on a species of *Urtica*. He compared *Botrytis Urticae* Libert (evidently a manuscript name) and *B. infestans* Mont. [= *Phytopthora infestans* (Mont.) de Bary], and concluded that the two were distinct. He failed, however, to characterize the former species adequately. Five years later, Berkeley and Broome (4) gave a more nearly complete description of *B. Urticae*. They described the conidia as "large, ovate, apex papillaeform," and gave the host as "common nettle" (undoubtedly referring to *U. dioica* L.).

Caspary (6) in 1855 transferred this species to the genus *Peronospora,* but failed to give a description or a reference to another description. Berkeley (3) in 1860 accepted this transfer and used the binomial *P. Urticae* Casp. for the fungus.

Next, de Bary (1), in his monograph of the family "Peronosporei," described a downy mildew found on *Urtica urens* L. which had apically rounded, basally pedicellate conidia. Oospores typical of his section Effusae were also observed. Although he applied the name *"Peronospora Urticae* (Lib.)" to his material, de Bary stated that Berkeley and Broome's description did not agree with his.

Subsequent workers, including Fischer (8), Berlese (5), Gäumann (9), and many others, consistently listed both species of *Urtica* as hosts of *P. Urticae.*

Salmon and Ware (12) noted zoospore germination of conidia produced on *U. dioica* and therefore made the combination *Pseudoperonospora Urticae* (Lib.) Salm. and Ware. After additional study, these workers (13) pointed out that two distinct downy mildews occur on species of *Urtica.* One, to which they applied the binomial *Pseudoper. Urticae* (Lib.) Salm. and Ware, has apically papillate and poroid, colored conidia which germinate by the formation of zoospores. It usually occurs on *U. dioica,* but has also been found on *U. urens.* The other, to which they apply the binomial *Per. deBaryi* Salm. and Ware, has apically rounded, basally pedicellate, colored conidia which germinate by means of a germ tube. This one occurs on *U. urens.*

In the United States, the first report of a downy mildew on *Urtica* was made in 1880 by Harkness and Moore (10), who applied the binomial *Per. Urticae* to it. Next, Trelease (15) reported the occurrence of *"Peronospora urticae* (Lib.)" on *Urtica gracilis* Ait. from two localities in Wisconsin. These two published records appear to be the only reports of a downy mildew on *Urtica* in the United States. The listings in Seymour's (14) and Wilson's (16) host indices are apparently based on these reports. In the light of the findings of Salmon and Ware, it seemed advisable to recheck all available material to determine the identity of the fungus present in the United States.

By personal correspondence, Lee Bonar has informed the writer that, except for a few type specimens, the collections made by Harkness and Moore were destroyed in the San Francisco earthquake and fire. It would appear that no portion of their collection is still extant.

Carroll W. Dodge of the Missouri Botanical Garden kindly loaned the author the following material: *"Peronospora Urticae* (Lib.) on *Urtica;* Kirkland (now Devil's Lake, Sauk Co.), Wis.; June, 1884. Pammel." The second collection, originally reported as collected at La Crosse, Wisconsin (15), is no longer represented

in the Trelease herbarium. The Kirkland specimen consists of two leaves abundantly covered on the lower surface with a cinereous mat of conidiophores.

An attempt to locate additional specimens proved unsuccessful. D. P. Rogers loaned the author two packets of the following collection: *"Peronospora Urticae* (Lib.) D By. on remains of *Aecidium Urticae* on *Urtica gracilis*. Glenwood, Minnesota; B. C. Taylor; July, 1891." A third portion of this collection reposes in the University of Wisconsin Cryptogamic Herbarium. Careful examination of all three specimens failed to disclose the presence of the downy mildew. Notes on the two packets from the New York Botanical Garden indicate that G. W. Wilson was also unable to find a downy mildew present.

Lee Bonar loaned the following specimen: *"Peronospora Urticae* D By. on *Urtica Lyallii;* Marysville, Wash.; July, 1927; J. M. Grant; U. Calif., Berkeley, Herb. No. 438288." Again a careful examination failed to reveal the presence of a downy mildew.

G. R. Hoerner, who has reported successful experimental infection of *Urtica* spp. with *Pseudoperonospora Humuli* (Miyabe & Takah.) Wilson from *Humulus Lupulus* L. (11), has informed the writer, in personal correspondence, that he has never found a downy mildew on any species of *Urtica,* and has no specimens of naturally infected species.

Since the collection by Pammel in the Trelease herbarium seems to be the only United States specimen extant, a description of that specimen is given below:

Peronospora deBaryi Salm. & Ware.   Figure 1

Foliicolous; infected area angular, becoming necrotic and light brown; thin, cinereous mat below; conidiophores hypophyllous, aseptate, hyaline or slightly tinted, emerging singly or in groups of 2–5 from a stoma, 4–6 times subdichotomously branched, 200–460 × 5–8 μ; crown about ½ the total height, main branches somewhat curved, arising at acute angles; ultimate branches acute to subacute, rather short, 4–9 μ, a few up to 15 μ long, the axial longer, straight or slightly incurved, the abaxial straight or occasionally slightly recurved; conidia light brown, broadly ellipsoid,

obtusely rounded apically, basally pedicellate, 19–27×13–19 μ (mean $22.42 \times 16.11 \mu$); oospores not observed.

On Urticaceae: Conidia on *Urtica gracilis* Ait.

Collected in Wisconsin in June.
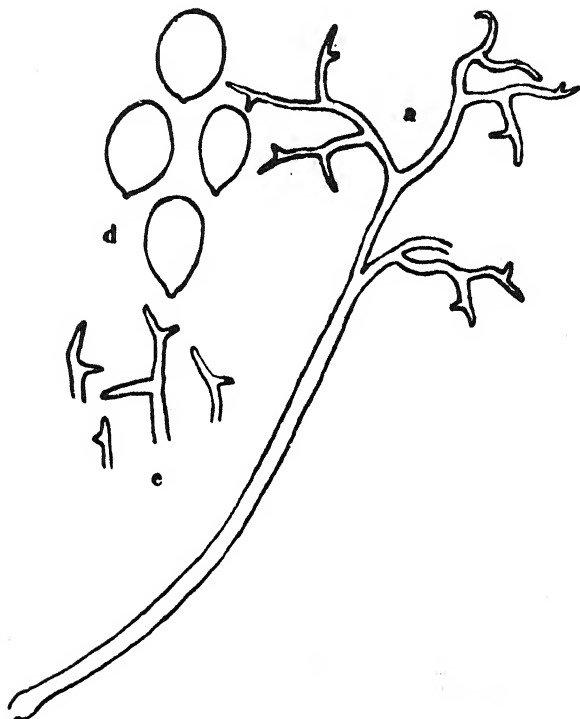
FIG. 1. *Peronospora deBaryi*—drawn with the aid of a camera lucida. *a,* conidiophore—$\times 300$; *d,* conidia—$\times 375$; *e,* ultimate branches—$\times 375$.

The above description agrees in all essentials with Salmon and Ware's description of *P. deBaryi*. The conidia of the American specimen are somewhat smaller than are those described by Salmon and Ware. Otherwise the American material is very similar. Because we have only one specimen, which lacks oospores, and because of the variability known to occur in the conidia of many downy mildews (7), the American material is at present considered to belong in *Peronospora deBaryi* Salm. and Ware.

The writer wishes to express his thanks to those who have loaned him specimens.

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THE HELMINTHOSPORIUM ON BUCHLOE DACTYLOIDES

C. L. Lefebvre and A. G. Johnson [1]

(WITH 3 FIGURES)

A characteristic species of *Helminthosporium* was collected on buffalo grass [*Buchloe dactyloides* (Nutt.) Engelm.] at Stockton, Kansas, on July 24, 1895, by Elam Bartholomew, his No. *1799*. He labelled the specimen (in Herb.) *"Helminthosporium inconspicuum* C. & E. var. *Buchloes* E. & B. n. var." Bartholomew supplied specimens of this same collection to J. B. Ellis who issued them in 1896 as extra numbers with Ellis and Everhart, North American Fungi, Century 34, as No. *45* (b), and with their Fungi Columbiani, Century 9, as No. *298* (b). The former is labelled *"Helminthosporium inconspicuum* C. E. var. *Buchloes,"* and the latter *"Helminthosporium inconspicuum* C. & E. var. *Buchloes."* Subsequently, this fungus has been referred to as *"H. inconspicuum* C. & E. var. *Buchloes* E. and Ev." (1, 2), *"H. inconspicuum buchloes* Ell. and Ev." (3), or simply as *"H. inconspicuum* var. *Buchloes"* (4).

In addition to examining the exsiccati specimens referred to above, six more recent collections of the same fungus on the same host, referred to later, have been examined. In all of these, the same characteristic fungus was found. Furthermore, the fungus was found to differ distinctly from that originally described on corn as *Helminthosporium inconspicuum* Cke. and Ell., now generally known as *H. turcicum* Pass. (5). The striking differences in the way the conidiophores are produced in the two fungi and in the shape, size, and hilum characters of their conidia are indicated in table 1, and differences in spore characters are shown in figures 2 and 3.

FIG. 1. Cross section of leaf of *Buchloe dactyloides* with groups of conidiophores and stromata of *Helminthosporium buchloes* between fibrovascular bundles. × 250.

TABLE 1

COMPARISON OF DIAGNOSTIC CHARACTERS OF *Helminthosporium turcicum* AND THOSE OF THE SPECIES OF *Helminthosporium* ON BUFFALO GRASS

Characters Compared	*Helminthosporium turcicum*	The *Helminthosporium* on Buffalo Grass
Conidiophores	Mostly in small groups, not on stromata.	Chiefly in large groups, on stromata.
Conidia		
Shape	Tapering about equally toward tip and base, base rather acute.	Tapering toward apex, base rounded.
Size	$15–25 \times 45–132\mu$	$8–11 \times 27–86\ \mu$
Hilum	Conspicuous, protruding.	Inconspicuous, not protruding.
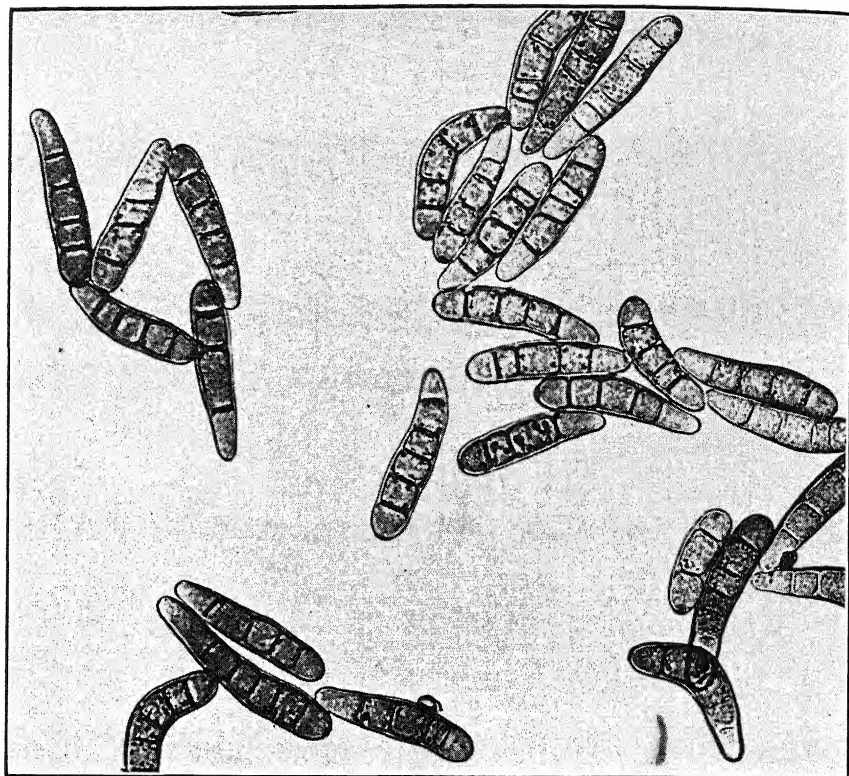
FIG. 2. Conidia of *Helminthosporium buchloes* developed in a moist chamber April 13, 1948, on an infected leaf of *Buchloe dactyloides* collected at Hays, Kansas, September 28, 1947. × 500.

Because of these differences, it seems clear that the fungus on buffalo grass does not belong as a variety under *H. inconspicuum* Cke. and Ell., but rather deserves specific rank. A search of the literature indicates that the variety *Helminthosporium inconspicuum* var. *buchloes* Ell. and Ev. has never been described. This name, therefore, is a *nomen nudum*. Likewise the name *"H. inconspicuum* var. *buchloes* E. & B."* (in Herb.) is a *nomen nudum*. Because of these considerations, the fungus on buffalo grass is here described as:

Helminthosporium buchloes sp. nov.

Maculis primum flavis vel rubro-flavis, demum stramineis ex parte obscuris; conidiophoris singulatim vel 2–20 caespitosis, olivaceo-brunneis,

apices versus pallidioribus, 2–9-septatis, 5–8 μ diam., 60–120 μ longis; conidiis subhyalinis usque pallide-fuliginosis in aetate obscurescentibus, 2–9-septatis, rectis curvatisve, interdum subsinuosis, ad apicem rotundatum attenuatis, basi rotundatis, hilo inconspicuo praeditis, tubulis duobus polaribus germinantibus, 8–11 μ diam., 27–86 μ longis.

In foliis *Buchloes dactyloidis* in U. S. A.
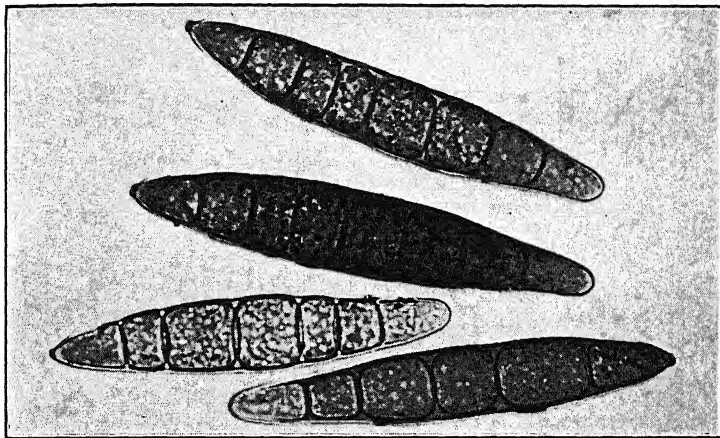
FIG. 3. Conidia of *Helminthosporium turcicum* Pass. (*H. inconspicuum* Cke. and Ell.) developed in a moist chamber June 2, 1948, on an infected leaf of corn (*Zea mays* L.) collected by Merle T. Jenkins near Cincinnati, Ohio, September 7, 1947. × 500.

Spots on leaves at first yellow to reddish-yellow, later bleaching to straw-color with dark blotches on both sides of leaf as stromata and conidiophores are produced. Conidiophores arising singly or in groups of two to twenty or more, the larger groups being produced from brown stromata between the fibrovascular bundles (FIG. 1); conidiophores olivaceous-brown, lighter toward apices, 2–9-septate, 5–8 μ in diameter at the basal septum, and 60–120 μ long; conidia subhyaline to light fuliginous becoming darker when older, 2–9-septate, straight or curved, occasionally tending to be somewhat sinuous, tapering toward the rounded apex, basal cell rounded, with hilum inconspicuous, germinating by two polar germ tubes, 8–11 × 27–86 μ (means 9.7 × 51.8 μ) (FIG. 2).

On leaves of *Buchloe dactyloides* (Nutt.) Engelm.

Collected by: Elam Bartholomew, Stockton, Kans., July 24, 1895; C. L. Lefebvre, Manhattan, Kansas, November 7, 1938; Mildred Pladeck, Maverick Co., Texas, August 11, 1939; R.

Sprague, Lincoln, Nebraska, September 12, 1940; C. L. Lefebvre, Woodward, Oklahoma, June 13, 1942; C. L. Lefebvre, Hays, Kansas, June 18, 1942 (type); J. L. Allison, Madison, Wisconsin. July, 1943; C. L. Lefebvre, Hays, Kansas, September 28, 1947.

Specimens of the type collection have been deposited in the Mycological Collections of the Bureau of Plant Industry, Beltsville, Maryland, and the Farlow Herbarium, Cambridge, Massachusetts.

PLANT INDUSTRY STATION,
    BELTSVILLE, MARYLAND

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NOTES AND BRIEF ARTICLES

The second in the new series of fungus forays of the Mycological Society of America was held at Highlands, North Carolina, September 2–7, 1947 under the leadership of Dr. Julian H. Miller, vice-president. Through the courtesy of Professor Thelma Howell, resident director, and the other officers, the Highlands Biological Laboratory was made available to those attending the foray as a base for collecting operations. Working space, driers, and other facilities were freely placed at the disposal of the mycologists.

On the afternoon of Sept. 2, Dr. L. S. Olive led the group along the trail to Sunset Rock on the Ravenel Estate with collecting along the way. September 3 was devoted to an all day trip to Rabun Bald, an interesting botanical area in Rabun County, Georgia. The mycological possibilities of a bog in which ecological studies were in progress were investigated enroute. An expedition to the Upper White Water Falls in Transylvania County occupied the morning of the 4th, and the two succeeding mornings were given over to collecting along Rhododendron and Kelsey Trails in the immediate vicinity of the Biological Laboratory. The afternoons were available for the study and care of the collections. As in the previous foray in West Virginia dry weather had prevented abundant production of fleshy forms, but sufficient fungi in other groups were found to make the occasion a success.

Dr. W. A. Campbell exhibited a most interesting series of kodachrome pictures of the larger fungi at the Laboratory on Wednesday evening, Sept. 3, and an equally instructive series of kodachromes of the local flora was presented on Friday evening. On Thursday afternoon the visiting ladies were taken by Professor Howell and her associates on a tour to High Hampton Inn and a number of estates in and about Highlands to view the floral effects.

The group as a whole attended an out-of-doors gathering at the Ravenel Estate during the early evening of September 5.

An informal dinner meeting was held at the Hotel Edwards on Thursday evening with approximately forty in attendance, including as guests Professor Howell and several of the Trustees of the Museum and Laboratory. The opportunity was improved upon to express the thanks of the Society and the members present for the courtesies and helpful cooperation extended by Professor Howell and her associates.

The following attended the foray: C. H. Arndt, Ernst Bessey, Lawrence G. Burk, Dr. and Mrs. H. H. Caldwell, W. A. Campbell, Edith K. Cash, A. Hugh Dempsey, W. W. Diehl, Chas. H. Driver, Marlin A. Espenshade, Francine E. Fisher, Dr. and Mrs. L. W. R. Jackson, Richard P. Korf, Morten and Bodil Lange, Daniel M. Lynch, Julian H. Miller, Dr. and Mrs. Lindsay S. Olive, Clark T. Rogerson, Dr. and Mrs. J. B. Routien, C. L. Shear, Dr. and Mrs Walter H. Snell, Wm. C. Snyder, Mr. and Mrs. John A. Stevenson, George E. Thompson, Mr. and Mrs. M. B. Walters, and Wm. H. Worrell, Jr. It is regretted that circumstances did not permit the taking of a group picture, always an interesting feature of previous forays.

A list of species collected in so far as reported follows, symbols as listed below being used to indicate the person who made the collections, or the institution in which they are deposited:

> (B) = Edith K. Cash, W. W. Diehl, C. L. Shear, John A.
>   Stevenson, Plant Industry Station, Beltsville, Md.
> (C) = R. P. Korf, C. T. Rogerson, Cornell University.
> (G) = J. H. Miller and associates, University of Georgia.
> (O) = L. S. Olive, Louisiana State University.
> (S) = Walter H. Snell, Brown University.

MYXOMYCETES: *Ceratiomyxa fruticulosa* (Muell.) Macbr. (O); *Fuligo septica* (L.) Weber (O); *Stemonitis* spp. (O).

PHYCOMYCETES: *Synchytrium aecidioides* (Pk.) Lagh. on *Amphicarpa monoica* (G).

ASCOMYCETES (except *Discomycetes*): *Anthostomella* sp. on *Solidago caesia* (G); *Balansia hypoxylon* (Pk.) Atk. on *Danthonia compressa, D. spicata* (B, G); *Bombardia fasciculata* Fr. on *Castanea dentata* (G); *Botryosphaeria ribis* (Tode ex Fr.) Gross.

& Dugg. on *Robinia pseudoacacia* (G) ; *Caliciopsis pinea* Pk. on *Pinus strobus* (B, G) ; *Ceratosphaeria* sp. (B) ; *Clypeolella leemingii* (Ell. & Ev.) Theiss. on *Galax aphylla* (B, G) ; *Cordyceps militaris* (L. ex Fr.) Lk. on insect larva (C) ; *C. ophioglossoides* (L. ex Fr.) Lk. on *Elaphomyces* sp. (C, G) ; *Creopus gelatinosus* (Tode ex Fr.) Lk. on *Rhododendron maximum* (G) ; *Cryptospora cinctula* (Cke. & Pk.) Sacc. on *Castanea dentata* (G) ; *Cucurbidothis pithyophila* (Fr.) Petr. on *Pinus strobus* (G) ; *Daldinia concentrica* Ces. & De N. (B) ; *Diatrype stigma* Hoffm. ex Fr. on *Betula* (B), on *Quercus alba* (G) ; *Diatrype asterostoma* Berk. & Curt. on *Nyssa sylvatica* (G) ; *Diatrypella favacea* (Fr.) Nits. (G) ; *Diaporthe leiphaemia* (Fr.) Sacc. var. *raveneliana* (Thuem. & Rehm) Wehm. on *Quercus alba* (G) ; *Diaporthe oncostoma* (Duby) Fckl. on *Robinia pseudoacacia* (G) ; *Elsinoë* sp. on *Cornus florida* (B) ; *Endothia parasitica* (Murr.) P. J. & H. W. Anderson on *Castanea dentata* (B, G, O) ; *Erysiphe polygoni* DC. on *Amphicarpa monoica, Baptisia tinctoria* (G) ; *Eutypella microcarpa* Ell. & Ev. on *Robinia pseudoacacia* (G) ; *Gibberella zeae* (Schw.) Petch on *Zea mays* (G) ; *Gnomonia* sp. on *Leucothoë catesbaei* (G) ; *Gnomonia setacea* (Pers. ex Fr.) Ces. & De N. on *Quercus maxima, Q. montana* (G) ; *Guignardia galactina* (Dearn. & House) Sacc. on *Galax aphylla* (G) ; *Hypocrea* sp. on *Acer* (B) ; *H. patella* Cke. & Pk. (B) ; *Hypoderma commune* (Fr.) Duby on Umbelliferae (B) ; *Hypodermopsis smilacis* (Schw.) Cash on *Smilax* sp. (B) ; *Hypomyces chrysospermus* Tul. on agaric (C) ; *H. hyalinus* (Schw.) Tul. on agaric (B, C) ; *Hypoxylon cohaerens* Pers. ex Fr. (B) ; *H. fuscum* Pers. ex Fr. on *Alnus rugosa* (G) ; *H. microplacum* (Berk. & Curt.) Miller on *Sassafras variifolium* (B, G) ; *H. multiforme* Fr. on *Betula lutea* (B) ; *H. punctulatum* (Berk. & Rav.) Cke. on *Quercus alba* (G) ; *H. rubiginosum* Pers. ex Fr. on *Betula lutea* (G), *Castanea dentata* (B) ; *H. serpens* Pers. ex Fr. on *Castanea dentata* (B), *Quercus alba* (G) ; *Leptosphaeria ogilviensis* (Berk. & Br.) Ces. & De N. (B) ; *L. vagabunda* Sacc. on *Azalea lutea* (G) ; *Lophodermium rhododendri* (Schw.) Pk. on *Rhododendron* sp. (B) ; *Melanomma pulvis-pyrius* (Pers. ex Fr.) Fckl. on *Castanea dentata* (G) ; *Metasphaeria* sp. on *Pinus strobus* (G) ; *Microsphaera alni* (DC.) Wint. on *Azalea*

viscosa (G); *M. alni vaccinii* (Schw.) Salmon on *Lyonia ligustrina*
(B); *Mycosphaerella* sp. on *Galax aphylla* (G); *M. leucothoës*
Miles on *Leucothoë catesbaei* (B, G); *Myiocopron smilacis* (De
N.) Sacc. on *Smilax* sp. (B), on *S. glauca* (G); *Myriangium
duriaei* Berk. & Mont. on scale insects on *Nyssa sylvatica* (G);
Nectria cinnabarina Tode ex Fr. on *Robinia pseudoacacia* (G);
N. coccinea Pers. ex Fr. on *Aronia melanocarpa, Castanea dentata*
(G); *N. ochroleuca* (Schw.) Berk. on *Amelanchier laevis* (B, C,
G); *Nectriella rousseliana* (Mont.) Sacc. (G); *Ophiodothella
vaccinii* Boyd on *Polycodium candicans* (G); *Ottwia hypoxylon*
(Ell. & Ev.) Shear (B); *Peckiella viridis* (Alb. & Schw.) Sacc.
(C); *Phyllachora lespedezae* (Schw.) Sacc. on *Lespedeza frutes-
cens* (G); *Phyllachora punctum* (Schw.) Orton on *Paspalum
dichotomum* (B); *Pleurage albicans* (Alb. & Schw.) Griff. (C);
Rosellinia clavariae (Tul.) Wint. on *Clavaria formosa* (G);
Sordaria appendiculata Auersw. (G); *Stigmatea rubicola* (Ell. &
Ev.) Theiss. on *Rubus flagellaris* (B, G), *R. hispidus* (G);
Uncinula salicis DC. ex Wint. on *Salix sericea* (G); *Ustulina
vulgaris* Tul. on dead wood (B); *Valsa ceratophora* Tul. on
Salix humilis (G); *V. pini* Alb. & Schw. ex Fr. on *P. strobus*
(G); *Xylaria ianthinovelutina* Mont. on *Magnolia fraseri* (G).

DISCOMYCETES (sensu latu): *Aleuria rhenana* Fckl. (C, G);
Aleurina atrovinosa (Cke.) Seaver (C); *Belonium carnulosum*
Rehm (C); *Chlorociboria aeruginascens* (Nyl.) Kanouse (B, C);
C. versiformis (Fr.) Seaver (C); *Chlorosplenium aeruginosum*
(Oed.) De N. (O); *C. chlora* (Schw. ex Fr.) Mass. on *Castanea
dentata* (G); *Coccomyces coronatus* (Schw. ex Fr.) Sacc. on
Rhododendron maximum (G); *Colpoma azaleae* (Schw.) Cke. on
Azalea lutea (G); *Dasyscypha nivea* Sacc. on *Quercus maxima*
(G); on dead coniferous wood (B, C); *Dermea balsamea* (Pk.)
Seaver on *Tsuga canadensis* (B, G); *Dermea puberula* Durand
on *Vitis* (B); *Durandiella fraxini* (Schw.) Seaver on *Fraxinus
americana* (G); *Galactinia michelii* Boud. (C); *Geoglossum
glabrum* Pers. ex Fr. (C); *G. simile* Pk. (B); *Glonium ab-
breviatum* (Schw.) Lohman on *Quercus coccinea* (G); *Godronia
rugosa* Ell. & Ev. on *Oxydendrum arboreum* (G); *G. urceolata*
(Ell.) Hoehn. on *Clethra acuminata* (B, G); *Helotium caudatum*

(Karst.) Vel. on *Amelanchier laevis* (B, G) ; *H. virgultorum* (Vahl ex Fr.) Karst. on dead wood (B) ; *Humaria scutellata* (L. ex Fr.) Fckl. (G) ; *H. setosa* (Nees ex Fr.) Fckl. (B) ; *H. umbrorum* (Fr.) Fckl. (C) ; *Hyaloscypha atomaria* (Starb.) Nannf. (C) ; *Hypoderma commune* (Fr.) Duby on *Solidago boottii* (G) ; *Lachnum cerinum* (Pers. ex Fr.) Nannf. on *Cornus florida* (B, G) ; *L. ciliare* (Schrad. ex Fr.) Rehm on *Quercus* sp. (B) ; *L. spiraeaicolum* (Karst.) Rehm (C) ; *L. virgineum* (Batsch ex Fr.) Karst. (B, C) ; *Leotia lubrica* Scop. ex Fr. (C, O) ; *L. viscosa* Fr. (C) ; *Lophodermium nitens* Darker on *Pinus strobus* (G) ; *L. pinastri* (Schrad. ex Fr.) Chev. on *Pinus rigida* (G) ; *Macropodia macropus* (Pers. ex Fr.) Fckl. (B) ; *Mollisia caesia* (Fckl.) Sacc. (C) ; *Mollisia cinerea* (Batsch ex Fr.) Karst. (B, C) ; *Ocellaria ocellata* (Pers. ex Fr.) Schroet. on *Salix humilis, S. sericea* (G) ; *Orbilia curvatispora* Boud. (C) ; *O. inflatula* Karst. (B) ; *O. leucostigma* Fr. (B, C) ; *O. luteo-rubella* (Nyl.) Karst. (C) ; *O. xanthostigma* Fr. (B, C) ; *Pestalopezia rhododendri* Seaver on *Rhododendron maximum* (B, C, G) ; *Pezicula* sp. on *Oxydendrum arboreum* (G) ; *P. alnicola* Groves on *Alnus rugosa* (G) ; *P. carnea* (Cke. & Ell.) Rehm on *Acer rubrum* (B, G) ; *Peziza badia* Pers. ex Fr. (B, C, G) ; *Pezizella lythri* (Desm.) Shear and Dodge on *Galax aphylla* (B) ; *Propolidium glaucum* (Ell.) Sacc. on *Castanea dentata* (B) ; *Rhytisma acerinum* Pers. ex Fr. on *Acer rubrum* (B) ; *R. andromedae* Fr. (C) ; *R. decolorans* Fr. on *Xolisma* (*Lyonia*) *ligustrina* (B, G) ; *R. salicinum* Pers. ex Fr. on *Salix humilis* (G) ; *R. vaccinii* Pers. ex Fr. on *Polycodium melanocarpum* (B) ; *Rutstroemia macrospora* (Pk.) Kanouse (C) ; *Schizoxylon* sp. on *Cornus florida* (G) ; *Stictis quercifolia* Cke. on *Quercus maxima* (B, G) ; *Tapesia fusca* (Pers. ex Fr.) Fckl. (B) ; *T. riccia* (Sacc.) Rehm (C) ; *T. torulae* Fckl. (C) ; *Trichoglossum walteri* (Berk.) Durand (C, G) ; *Tympanis pithya* Karst. on *Pinus strobus* (G).

BASIDIOMYCETES :

Uredinales: Coleosporium helianthi (Schw.) Arth. on *Helianthus giganteus, H. tuberosus* (B, O) ; *C. inconspicuum* (Long) Hedgc. on *Coreopsis major* (B) ; *C. oemleri* (C) ; *C. solidaginis* (Schw.) Thuem. on *Solidago* sp. (O) ; *S. arguta* (B), *S. bicolor*

(B), *S. caesia* (G), *S. conferta* (G), *S. curtisii* (B), *S. monticola* (G), *S. rugosa* (G), *S. tomentosa* (B) ; *C. vernoniae* Berk. & Curt. on *Vernonia noveboracensis* (B, G) ; *Cronartium quercuum* (Berk.) Miy. on *Quercus coccinea* (G) ; *Frommea obtusa* (Str.) Arth. on *Potentilla canadensis* (G) ; *Gymnosporangium juniperi-virginianae* Schw. on *Juniperus virginiana* (O) ; *G. nidus-avis* Thaxt. on *J. virginiana* (O) ; *Kuehneola uredinis* (Lk.) Arth. on *Rubus argutus* (B, C, G) ; *Melampsora abieti-capraearum* Tub. on *Salix humilis* (B) ; *Puccinia angustata* Pk. on *Scirpus georgianus, S. sylvaticus* (G) ; *P. bolleyana* Sacc. on *Carex lurida* (G) ; *P. coronata* on *Phleum pratense* (G) ; *P. helianthi* Schw. on *Helianthus tomentosus* (G) ; *P. menthae* Pers. on *Koellia beadlei* (G) ; *P. mesomajalis* Berk. & Curt. on *Clintonia borealis* (B, C) ; *P. rubigo-vera* (DC.) Wint. var. *impatientis* (Arth.) Mains on *Agrostis perennans* (G) ; *P. smilacis* Schw. on *Smilax* sp. (B) ; *P. tenuis* (Schw.) Burr. on *Eupatorium urticaefolium* (B) ; *P. violae* (Schum.) DC. on *Viola primulifolia* (B, G) ; *Pucciniastrum hydrangeae* (Berk. & Curt.) Arth. on *Hydrangea arborescens* (B, C, G, O), *H. cinerea* (B) ; *P. myrtilli* (Schum.) Arth. on *Azalea* sp. (B, C, O) ; *Uredinopsis macrosperma* (Cke.) Magn. on *Pteridium aquilinum* (O) ; *Uromyces hedysari-paniculati* (Schw.) Farl. on *Desmodium canescens* (B, C, G).

Tremellales: *Calocera cornea* (Fr.) Lk. on *Rhododendron maximum* (B) ; *Dacrymyces minor* Pk. (O) ; *D. palmatus* (Schw.) Bres. (O) ; *Exidia nucleata* (Schw.) Burt (O) ; *E. recisa* (S. F. Gray) Fr. (O) ; *Guepinia spathularia* (Schw.) Fr. (O) ; *Sebacina incrustans* Pers. ex Tul. (B) ; *S. podlachica* Bres. (O) ; *Tremellodendron candidum* (Schw.) Atk. (B, O) ; *T. merismatoides* (Schw.) Burt (B, O) ; *T. tenax* (Schw.) Burt (O) ; *Tremellodon gelatinosum* (Pers.) Fr. (O).

Homobasidiomycetes (ex parte, see also the following paragraphs) : *Aleurodiscus candidus* (Schw. ex Fr.) Burt on *Quercus alba, Q. borealis* (B) ; *Amanita caesarea* Fr. (B, O) ; *A. muscaria* L. ex Fr. (O) ; *A. rubescens* Fr. (B) ; *A. solitaria* Fr. (S) ; *Armillaria aurantia* Fr. (S) ; *Calodon geogenium* (Fr.) Karst. (B, S) ; *C. scrobiculatum* (Fr.) Quél. (B) ; *C. velutinum* (Fr.) Quél. (S) ; *C. zonatum* (Batsch ex Fr.) Quél. (B) ; *Cantharellus floccosus*

Schw. (B); *Clavaria mucida* Pers. (O); *Clitocybe clavipes* Fr.
(B); *C. illudens* Schw. (B); *Corticium roseum* Pers. ex Fr. on
Populus tremuloides (G); *Craterellus cornucopioides* L. ex Fr.
(B); *Cyclomyces greenei* Berk. (C); *Dentinum albidum* (Pk.)
Snell (S); *D. repandum* (L. ex Fr.) S. F. Gray (S); *Fistulina
hepatica* Huds. ex Fr. (B); *Fomes applanatus* (Pers. ex Fr.)
Gill. (B); *Hydnum fennicum* (Karst.) Sacc. (B, S); *Hymenochaete arida* Karst. on *Cornus florida* (B); *H. corrugata*
(Fr.) Lév. on *Acer rubrum* (B); *H. fuliginosa* (Pers.) Bres. on
Rhododendron maximum (B); *Lactarius subpurpureus* Pk. (S);
L. volemus Fr. (S); *Lenzites betulina* L. ex Fr. (B); *L. saepiaria*
Wulf. ex Fr. on *Tsuga canadensis* (B); *Marasmius* sp. on *Tsuga
canadensis* (B); *Panus stypticus* Fr. on *Rhododendron maximum*
(B); *Peniophora sanguinea* (Fr.) Bres. on *Alnus* (B); *P. subalba*
(Burt) Rogers & Jackson on *Alnus* (B); *Pholiota caperata* Fr.
(S); *Phylloporus rhodoxanthus* (Schw.) Bres. (S); *Polyporus
abietinus* Dicks. ex Fr. on *Picea rubens* (G); *P. adustus* Willd. ex
Fr. on *Acer rubrum* (G); *P. berkeleyi* Fr. (B); *P. cinnabarinus*
Jacq. ex Fr. (B); *P. cinnamomeus* Jacq. ex Fr. (B, S); *P. elegans*
Bull. ex Fr. (B); *P. focicola* Berk. & Curt. (S); *P. giganteus*
Pers. ex Fr. (B, G); *P. hirsutus* Wulf. ex Fr. (B, O); *P. pargamenus* Fr. on *Acer rubrum* (B); *P. perennis* L. ex Fr. (C); *P.
sulphureus* Bull. ex Fr. (C); *P. tulipiferus* (Schw.) Overh. on
Castanea dentata (B); *P. versicolor* L. ex Fr. on *Populus tremuloides* (G), *Rhododendron maximum* (B), *Sassafras officinale*
(B), dead wood (O); *Poria* sp. on *Acer rubrum* (G); *P. isabellina* (Fr.) Overh. on *Rhododendron maximum* (B); *P. versipora*
(Pers. ex Fr.) Romell on *R. maximum* (B); *Russula crustosa*
Pk. (S); *R. emetica* Fr. (O); *R. foetans* Fr. (S); *R. variata*
Bann. & Pk. (S); *R. virescens* Fr. (S); *Schizophyllum commune*
Fr. (O); *Solenia anomala* (Pers. ex Fr.) Fckl. on *Betula lutea*
(G); *S. fasciculata* Pers. on *Alnus* (B); *Steccherinum ochraceum*
Pers. ex S. F. Gray on *Castanea dentata* (B); *Stereum frustulatum*
(Pers. ex Fr.) Fr. on *Quercus* sp. (G); *S. hirsutum* Willd. ex Fr.
on *Acer rubrum* (B); *S. lobatum* (Kunze) Fr. (O); *S. rameale*
Schw. on *Nyssa sylvatica* (B), *Rhododendron maximum* (B),
dead wood (O); *Thelephora vialis* Schw. (S); *Tomentella coriaria*

(Pk.) Bourd. & Galz. on *Rhododendron maximum* (B); *Tricholoma equestre* Fr. (S).

BOLETACEAE (All collections by Dr. W. H. Snell unless otherwise indicated): *Boletellus betula* (Schw.) Gilbert; *Boletinus pictus* Pk. (B, S); *Boletus bicolor* Pk.; *B. edulis* Bull. ex Fr.; *B. ferrugineus* Frost; *B. fumosipes* Pk.; *B. griseus* Frost; *B. illudens; B. miniato-olivaceus* Frost; *B. modestus* Pk.; *B. nobilis* Pk.; *B. peckii* Frost; *B. separans* Pk.; *B. vermiculosus* Pk.; *Gyroporus castaneus* (Bull. ex Fr.) Quél.; *Leccinum chromapes* (Frost) Singer; *L. nigrescens* (Rich. & Roze) Singer; *L. rubropunctum* (Pk.) Singer; *L. rugosiceps* (Pk.) Singer; *L. scabrum* (Bull. ex Fr.) S. F. Gray; *Porphyrellus gracilis* (Pk.) Singer; *Pulveroboletus auriflammeus* (Berk. & Curt.) Singer; *P. auriporus* (Pk.) Singer; *P. ravenelii* (Berk. & Curt.) Singer; *P. retipes* (Berk. & Curt.) Singer (B, S); *Strobilomyces floccopus* (Vahl ex Fr.) Karst. (O, S); *Suillus granulatus* (L. ex Fr.) Kuntze ssp. *snellii* Singer; *S. punctipes* (Pk.) Singer; *S. subaureus* (Pk.) Snell; *Tylopilus alboater* (Schw.) Murr.; *T. eximius* (Pk.) Singer; *T. felleus* (Bull. ex Fr.) Karst.; *Xanthoconium affine* (Pk.) Singer; *Xerocomus subtomentosus* (L. ex Fr.) Quél. From near Winston-Salem, No. Car.: *Strobilomyces confusus* Singer, *Tylopilus felleus* var. *minor* Coker & Beers.

GASTEROMYCETES: *Calostoma cinnabarina* Desv. (B); *Crucibulum vulgare* Tul. (B); *Lycoperdon atropurpureum* Vitt. (B); *L. echinatum* Pers. (C); *L. gemmatum* Batsch (B); *L. pyriforme* Schaeff. (O); *L. subincarnatum* Pk. (B); *Scleroderma geaster* Fr. (S); *S. lycoperdoides* (Schw.) (C).

FUNGI IMPERFECTI: *Aegerita epixylon* DC. ex Duby on *Castanea dentata* (B); *Cercospora dioscoreae* Ell. & G. Martin on *Dioscorea villosa* (G); *C. omphacodes* Ell. & Holw. on *Phlox* sp. (B); *C. rubi* Sacc. on *Rubus flagellaris* (G); *C. smilacina* Sacc. on *Smilax bona-nox* (B); *C. smilacis* Thuem. on *Smilax glauca* (B, G); *C. venturioides* Pk. on *Asclepias syriaca* (G); *C. vexans* C. Massal. on *Fragaria virginiana* (G); *Chondropodiella clethrincola* (Ell.) Hoehn. on *Clethra acuminata* (B, G); *Cladosporium epiphyllum* Pers. ex Fr. on *Robinia pseudoacacia* (G); *C. gloeosporioides* Atk. on *Hypericum mutilum* (G); *Colletotrichum trichellum* (Fr.)

Duke on *Hedera helix* (B); *Cylindrosporium acerinum* Tracy & Earle on *Acer pennsylvanicum* (B); *Cytospora pinastri* Fr. on *Abies fraseri* (G); *Darluca filum* (Biv.) Cast. on *Coleosporium solidaginis* and other Uredinales (B, C); *Discosia artocreas* (Tode ex Fr.) Fr. on *Epigaea repens* (B); *Gloeosporium caryae* Ell. & Dearn. on *Carya glabra* (G); *Graphium hamamelidis* Van Hook on *Hamamelis virginiana* (B, G); *Helminthosporium* sp. on *Phleum pratense* (C); *Hendersonia heloniaefolia* (Cke. & Ell.) Cke. on *Helonias* sp. (B); *Isariopsis clavispora* (Berk. & Curt.) Sacc. on *Vitis bicolor* and *Vitis* sp. (B, G); *Macrophoma smilacis* (Pk.) Berl. & Vogl. on *Smilax glauca* (G); *Monochaetia desmazierii* Sacc. on *Castanea dentata* (B); *Monotospora megalospora* Berk. & Br. on *Castanea dentata* (B); *Penicillium camemberti* Thom on agaric (B); *Penicillium thomii* Maire (C); *Pestalotia funerea* Desm. on *Thuya occidentalis* (G); *Phyllosticta epigaeae* Pk. on *Epigaea repens* (B); *Sepedonium brunneum* Pk. (C); *Sepedonium chrysospermum* (Bull.) Lk. ex Fr. (B); *Septocylindrium concomitans* (Ell. & Holw.) Halst. on *Bidens frondosa* (G); *Septoria cornicola* Desm. on *Cornus alternifolia* (G); *S. violae* West. on *Viola primulifolia* (B, G); *Septoria* sp. on *Zanthorhiza apiifolia* (G); *Sphaceloma plantaginis* Jenkins & Bitanc. on *Plantago rugelii* (B); *Sphaerographium* sp. on *Azalea arborescens* (G); *Sporocybe azaleae* (Pk.) Sacc. on *Rhododendron maximum* (B, C); *Sporodesmium antiquum* Cda. (B); *Sporotrichum quercuum* Shear on *Quercus* sp. (B); *Stilbella ostracogena* Cda. on *Castanea dentata* (B); *Volutella* sp. on *Magnolia fraseri* (G).—John A. Stevenson.

Plant Industry Station,
Beltsville, Maryland

Proposals for Amendment of the International Rules

I

Art. 47. An alteration of the diagnostic characters or of the circumscription of a group **without exclusion of the type** does not warrant the citation of an author other than the one who first published its name.

Proposal: delete the words "without exclusion of the type."

Argument: The sentence quoted is the first and essential sentence in Art. 47 as amended at Amsterdam (Bisby, G. A., An introduction to the taxonomy and nomenclature of fungi, p. 81. 1945; Brittonia 6: 16. 1947). The phrase in bold-face is the modification (Zesde Int. Bot. Congr. Amsterdam Proc. 1: 344. 1936). It, and with it the whole sentence, is ambiguous in the extreme. If the sentence means anything, it implies the affirmative statement: **An alteration of the diagnostic characters or of the circumscription of a group with exclusion of the type warrants the citation of an author other than the one who first published the name.** Since "a nomenclatorial type is that constituent element of a group to which the name is permanently attached" (Art. 18), such exclusion would seem to be excluded by the definition of "type." Under such an interpretation, which is the logical one, the added phrase is inoperative and achieves no end save that of confusion, and clearly should be revoked. But it is likely that the implications of the type concept will be overlooked and the amended article illogically accepted, although often under protest, as implying the positive paraphrase just given.

The author of a name is cited "for the indication of the name of the group to be accurate and complete" (Art. 46). After the incorporation of the type concept into the rules, and before the addition of the phrase under discussion, the author was the one "who first published the name in question" (Art. 46). Knowing the name and its author, a taxonomist was at least well on the way to learning the date of the name (for consideration of priority), and the type, or at least, the constituent elements eligible as lectotype (for determining the application of the name). This is not to say that either could always be "readily verified"; but at least the field of investigation was greatly narrowed. Homonyms could give rise to difficulties; but a straightforward and honest homonym could be dealt with by the "Auct. II non Auct. I" formula widely used long before it was adopted as Rec. XXXII bis (Bisby, l.c., p. 83; Brittonia 6: 18; Amsterdam Proc. 1: 344); homonyms were identical names independently published for different types.

But under the amended Art. 47, according to the interpretation

under discussion, there are as many homonyms of any name as there are uses of that name with "alteration of the diagnostic characters or of the circumscription of a group [with] exclusion of the type." · The first two points are easy to identify. Since a later author rarely repeats exactly in his description the original characters ascribed to a group, a majority of subsequent descriptions embody "alteration of the diagnostic characters." One need scan the successive descriptions of an early genus in only a few examples to make sure of that: *Agaricus* L. ex Fr. was first validly published with "lamellae . . . ascigerae," but later described as possessing basidia, and most recently as annulate and with purple-brown spores. *Puccinia* was first validly described as having spores "septulis distinctis"; additional characters have repeatedly been added, as that the spores are one-septate (excluding multi-septate species) and that the telia are dry (excluding those with gelatinous horns). Since a later author usually adds to or subtracts from the number of original species, either incidentally or systematically, the majority of subsequent treatments embody "alteration of the . . . circumscription of a group." *Agaricus* has been both repeatedly enlarged, by the addition of new species. and repeatedly narrowed, by exclusion of one component after another as autonomous genera, and finally has been restricted to Ser. *Pratella* Trib. *Psalliota*. *Puccinia* has similarly been enlarged, and similarly narrowed by the exclusion of *Phragmidium, Gymnosporangium, Tranzschelia,* etc. It is clear that such alterations of the diagnostic characters and circumscription do not warrant the citation of an author other than the one who first published the name—unless (according to the interpretation under discussion) accompanied by exclusion of the type.

Assuming without prejudice that the type can be excluded, how can it be done? It is here that the sentence under discussion is completely ambiguous. There are many possible ways of excluding the type: 1. By description. Clements & Shear described *Tulasnella* (in a key: Gen. Fung. 158) as having "basidia . . . cruciately divided by 3 vertical septa," thus excluding *T. lilacina,* the genotype. 2. By a statement of geographic range which excludes the type species or specimen. 3. By designation of another type. Clements & Shear designated *T. anceps,* an alien species, as

the type of *Tulasnella* (l.c., p. 342).   4. By exclusion of the type species (etc.) from the subgenus (etc.) named *Typicae, Eu-,* or otherwise marked as the typical subdivision.   Saccardo (Syll. **4**: 283–288) excluded both original species of *Zygodesmus* from I. *Eu-Zygodesmus,* assigning them to II. *Hypochniella* B. *Leiosporae* of *Zygodesmus.*   5. By relegating the type to a list of *Ignotae, Dubiae,* etc., having adequately described other components of the group.   6. By various other phrases, comments, or discussions indicating that the characters and circumscription in a particular treatment were derived chiefly from other elements of the group than the type.   7. By omission of the type.   When an author discusses a genus as it occurs in Italy, he omits its type if that occurs only in Germany.   When an author enumerates material of a species collected in South America, he may omit its type if that was collected in the Caribbean.   8. By assignment or transfer of the type to another group.   Desmazières wrote (Ann. Sci. Nat. iii **11**: 279) : "Le *Phoma pustulata,* Fr., [an erroneous spelling, but undoubtedly the designated type of *Phoma*] est un *Sphaeria."* Sparrow (Aquatic Phycomycetes) assigned all 16 original species of *Phlyctidium* to other genera.   Saccardo (Syll. **2**: 285) transferred both original species of *Venturia* to *Pyrenophora.*   Each of these authors nevertheless used the generic name in question for other species.

It is unlikely that any author would pounce upon all these possibilities as constituting veritable exclusion of the type, and likely that most taxonomists, if they allowed the possibility that the type could be excluded, would accept only the last : explicit exclusion, or explicit assignment of the type to another group.   Nevertheless, since none of them is eliminated by Art. 47, nor by any other rule (unless all are eliminated by Art. 18), Art. 47 is at present insuperably ambiguous.

Supposing the interpretation under consideration to be the accepted one, what are the effects of the interpolation "without exclusion of the type"?   They are two : 1. The bringing into existence of a number of validly published homonymous groups where only one stood before.   The number depends, of course, on what is held to constitute exclusion of the type.   Such groups will clutter up the synonymy, and greatly increase the labors of tax-

onomists, but can and will be disregarded by most. 2. The creation of the possibility of recognizing a name *in any sense in which it has been misapplied* (if exclusion of the type can be plausibly argued) and, if the name is that of a genus, of conserving it.   That seems to be the one use of the amendment and all the trouble it entails, and the one occasion for its adoption: a device to permit the retention, by a change in author, of generic names which have been incorrectly used.   It is one more evasion of the type concept, which has proved so useful to many botanists and so distasteful to a few.   As such it is burdensome, confusing, and inaccurate.   The modification is "on trial until the next Congress" (Art. 74), which should reject it.   Art. 18 contains the sound and practical provision for such cases: "The name of a group must be changed if the type of that name is excluded."

II

Proposal: To adopt, as a part of Appendix I, the following

REGULATIONS FOR FIXING GENERIC TYPES

I. RULES

1. The type species shall be the species or one of the species included in the genus when first validly published after the applicable date set forth in Art. 20.   If a genus included but one species when originally published, that species is the type.   The types of genera adopted through citations of literature published before the starting-point for the group in question (with or without change of name) shall be selected from those of the original species which received names in the first legitimate publication.

2. When, in the original publication of a genus, one of the species was definitely designated as type, that species shall be accepted as the type, regardless of other considerations.   If *typicus* or *typus* was used as a new specific name for one of the species, that species shall be accepted as the type as if it were definitely designated.

3. The publication of a new generic name as an avowed substitute for an earlier one does not change the type of the genus.

4. If a genus without an originally designated type contains among its original species one with the generic name used as a specific name, either as an accepted name or synonym, that species is to be accepted as the type.

5. If a genus when originally published included more than one species, and no species was definitely designated as type, nor indicated according to Paragraph 4, the choice of the type shall accord with the following principles:

(a) Species inquirendae or species doubtfully referred to the genus or mentioned as in any way exceptional are to be excluded from consideration in selecting the type.

(b) Species which definitely disagree with the generic description (provided others agree), or which possess characters stated in the generic description as rare or unusual, are to be excluded from consideration in selecting the type.

6. Among species equally eligible, the preference shall be given to the first known to have been designated as the type.

II. RECOMMENDATIONS

In the selection of types of genera previously published, but of which the type would not be indicated by the preceding regulations, the following points are to be taken into consideration:

i. The type species should usually be the species or one of the species which the author had chiefly in mind. This is often indicated by

(a) A closer agreement with the generic description.

(b) Certain species being figured (in the same work).

(c) The specific name, such as *vulgaris, communis, medicinalis* or *officinalis*.

ii. The type species should usually be the one best known to the author. It may be assumed that an indigenous species (from the standpoint of the author), or an economic species, or one grown in a botanical garden and examined by the author, would usually represent an author's idea of a genus.

iii. The preceding conditions having been met, preference should be shown for a species which will retain the generic name in its most widely used sense, or for one which belongs to a division of

the genus containing a larger number of species, or for the historically oldest species.

iv. If it is impossible to select a type under the conditions mentioned above the first of equally eligible species should be chosen.

Discussion: The present Rules are somewhat prolix in their provisions for determining the proper names for plants, but quite vague and unsatisfactory in indicating for what plants these names are to be used. The **type method** was written into the Rules (Art. 18; Rec. IV–VII) in response to the demand for regulations covering the applications of names; but perhaps out of fear that it would invalidate current irregular or perverted usages, Appendix I, "Regulations for determining types," has not been written. In fact, the heading for this Appendix bears the note "No draft of this Appendix has been submitted."

Whether or not such a draft has been formally "submitted," such drafts, and excellent ones, have been prepared and published. The one here proposed is essentially a rearrangement of one written by a committee of the Botanical Society of America, composed of A. S. Hitchcock (Chairman), N. L. Britton, and J. M. Greenman, with the collaboration of Leroy Abrams and Witmer Stone, and published in Science **49**: 333–335. 1919. It has the advantages of being clear, simple, and honest, and the further advantage of embodying established custom (Art. 5)—that is, the consistent practice of most botanists (and of nearly all botanists when they are not seeking justification for the employment of a beloved, but illegitimate nomenclature). If nomenclature is to be governed by rules rather than exception, Appendix I should no longer be left a blank; and these provisions recommend themselves as filling a pressing need.—Donald P. Rogers.

New York Botanical Garden

Special Committee on Fungi for the International Botanical Congress at Stockholm.—At the International Conference on Botanical Nomenclature held at Utrecht, Netherlands, in June 1948, additional members were added to the special committee on fungi in order to broaden and expedite the work necessary to prepare for the Stockholm Congress. New appointees were J. Westerdijk (Centraalbureau v. Schimmelcultures, Baarn,

Netherlands), D. P. Rogers (N. Y. Bot. Gard., Bronx Park, New York, N. Y.), C. W. Emmons (Nat. Inst. Health, Bethesda, Md.), G. W. Martin (Univ. Iowa, Iowa City, Iowa), R. Singer (Inst. Miguel Lillo, Calle Miguel Lillo, Tucuman, Argentina), S. P. Wiltshire (Commonwealth Mycol. Inst. Kew, Surrey, England), M. Le Gal (6 Rue Chomel, Paris VI, France), M. A. Donk (Herb. & Mus. Syst. Bot., Buitenzorg, Java), and B. B. Mundkur (Indian Agr. Res. Inst., New Delhi, India). Previously appointed members of this committee are C. L. Shear (2253 No. Madison St., Arlington, Va.), K. B. Boedijn (Herb. & Mus. Syst. Bot., Buitenzorg, Java), R. Ciferri (Bot. Inst. & Ital. Crypt. Lab., Univ. Pavia, Pavia, Italy), R. Maire (3 Rue Linné, Algiers, Algeria), J. A. Nannfeldt (Uppsala Univ. Inst. Syst. Bot., Uppsala, Sweden), F. Petrak (Naturhist. Mus., Wien I, Burgring 7, Austria), F. J. Seaver (N. Y. Bot. Gard., Bronx Park, New York, N Y.), E. M. Wakefield (Royal Bot. Gard., Kew, Surrey, England), A. M. Bottomley (Dept. Agr. Pretoria, Union S. Africa), W. J. Lütjeharms (Univ. Coll., O. F. S., Bloemfontein, Union S. Africa), J. Ramsbottom (Brit. Mus., Cromwell Road, London, S. W. 7, England), A. Trotter (R. Ist. Super. Agr. Portici, Naples, Italy), and W. H. Weston (Harvard Univ., Divinity Ave., Cambridge, Mass.). T. A. Sprague (care of Royal Bot. Gardens, Kew, Surrey, England), and J. Lanjouw (Bot. Mus. & Herb., 106 Lange Niewstraat, Utrecht, Netherlands) are "ex officio" members of all committees.

It is the duty of this committee to consider all proposals to modify in any way the present International Rules of Botanical Nomenclature in so far as fungus nomenclature is concerned. Each specific proposition should be submitted with at least two copies to each member of the committee. Each member will carefully consider each proposal and indicate clearly whether for or against it and return one marked copy to the secretary as promptly as possible in order that the results may be compiled and made ready for the Congress. It is hoped that this notice will be accepted by committee members in lieu of a personal letter and that we may have the fullest cooperation in carrying out the work.— C. L. Shear, Secretary, 2253 No. Madison St., Arlington, Virginia, U. S. A.
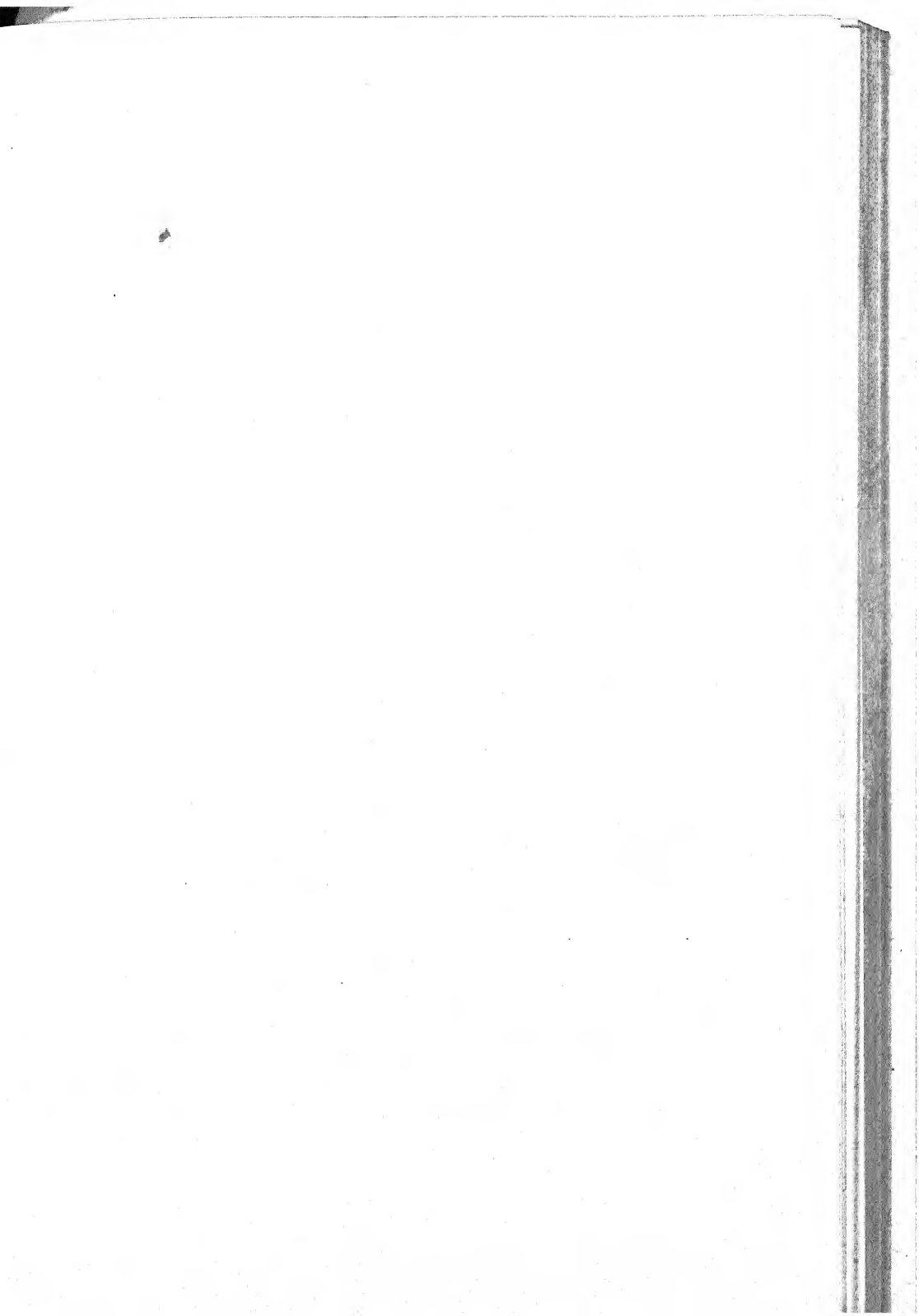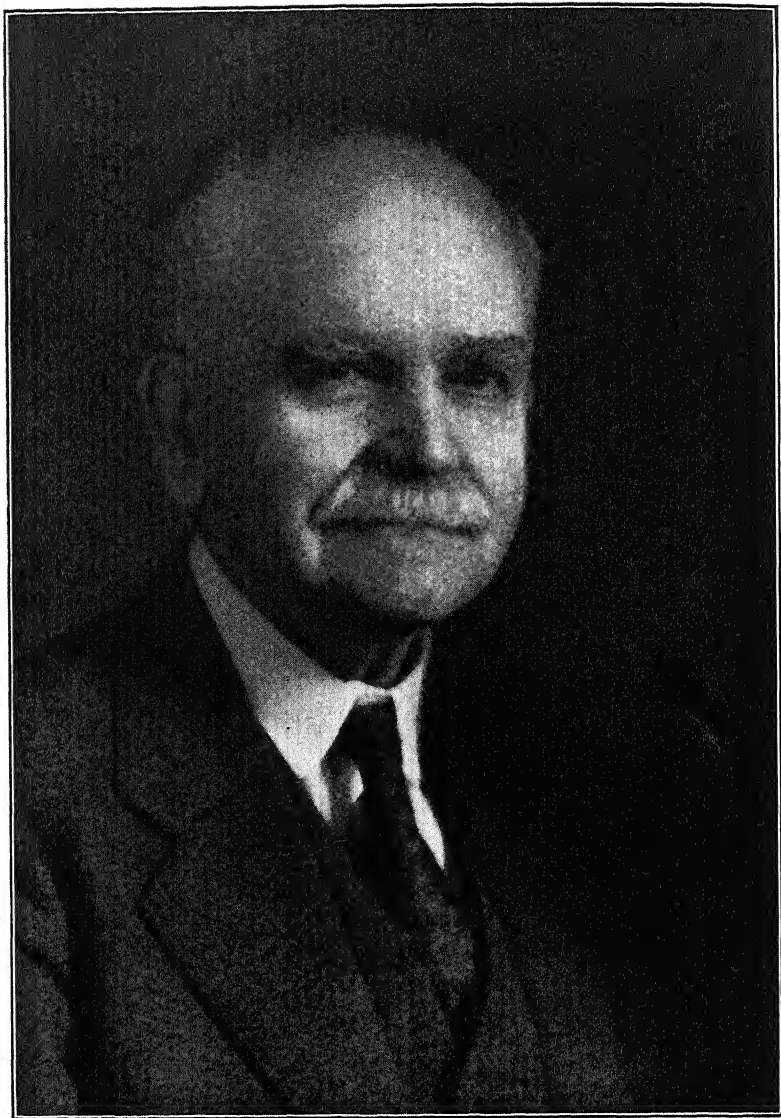
WILLIAM HENRY LONG, 1867–1947

WILLIAM HENRY LONG, 1867–1947

JOHN A. STEVENSON

William Henry Long was born March 7, 1867, in Navarro Co., Texas, and died December 10, 1947 at Albuquerque, New Mexico, where he had long resided. He took his undergraduate work at Baylor University, Waco, Texas, receiving the A.B. degree in 1888. Following graduation he served as Professor of Natural Sciences with his Alma Mater until 1892 when he assumed a similar position at Burleson College, Greenville, Texas.

Feeling the need for further botanical training he severed his connection with this institution in 1899 to take graduate work in his chosen field at the University of Texas, under the guidance of Professors Wm. L. Bray and W. M. Wheeler, where he also served as assistant in botany for the period 1900–1901. As a result of his studies here the A.M. degree was given him in 1900. His first mycological paper on the distribution of the fungi of Austin and vicinity was prepared and published at this time. For the next nine years he was Professor of Botany at North Texas State Normal College at Denton. Here with increased tempo he continued his mycological studies with emphasis on the Uredinales. During this time he published his classical paper on the Ravenelias of the United States and Mexico as well as several on other rusts and on the Phalloideae of Texas.

Throughout his career Long was an avid collector of fungi, and very early in his scientific work commenced assembling an herbarium both by personal collecting and by exchange. Unfor-

tunately, most of his Texas specimens were lost in a fire in his home at Denton about 1907. Many of his collections, however, had been shared with other mycologists so that the loss was not as serious as it would have been had it not been for this life-long habit of collecting abundantly and exchanging freely.

During this time, in addition to extensive mycological field work in the vicinity of Denton, several summers were spent at Cornell University in similar activities under the direction of Professor Geo. F. Atkinson. These studies were ultimately the basis of his doctoral dissertation presented in 1917 to the University of Texas which granted the Ph.D. degree.

Long entered federal service in January, 1910 as a member of the editorial staff of the Experiment Station Record in the Department of Agriculture at Washington, being assigned to the field of "agricultural botany, bacteriology and vegetable pathology." This sedentary existence was not particularly to his liking and after some 18 months he transferred to the then comparatively new Office of Forest Pathology in the Bureau of Plant Industry. Here under the leadership of Haven Metcalf he became one of the group, which included George G. Hedgcock, P. Spaulding, E. P. Meinecke, J. R. Weir and others, who pioneered in the development of forest disease work in the United States.

Following a short stay at Washington headquarters where he studied wood rots, and forest-tree rusts, particularly those of the genus *Gymnosporangium,* he was assigned to head up the work in the Southwestern States with headquarters at Albuquerque, New Mexico. Here he spent the remainder of his career, for a time covering a territory which extended from Arizona to Florida, but soon restricting his activities to Arizona, New Mexico, and Colorado. Some forty technical papers cover his work for the period, ranging over the many problems encountered in the forests of his region, all studied in close cooperation with the U. S. Forest Service.

In addition to continued work on forest-tree rusts and wood rots in general, his outstanding accomplishments were the development of brush disposal methods on national forests, initiation of sylvicultural methods of handling yellow pine stands infested by

mistletoe, and studies on the fungi which rot railroad ties, fence posts, and poles, aimed at preventing their attack.

Following his retirement in July, 1937, he took up with great enthusiasm what had been one of his mycological hobbies for many years, a study of the taxonomy of the Gasteromycetes. In this work he gave particular attention to those of the Southwest and with the assistance of David J. Stouffer and other experienced collectors, brought together a very extensive and valuable series of these fungi. The results of Long's studies are for the most part in the series of papers under the general title, Studies in the Gasteromycetes. His private herbarium was bequeathed to the Smithsonian Institution.

Dr. Long was a member of several technical organizations during his active career, among which were the Mycological Society of America, the American Phytopathological Society, the Texas Academy of Science, the Ecological Society of America, the Botanical Society of Washington.

There follows a selected list of his writings in Mycology and Phytopathology. Papers in other fields, as well as abstracts and popular articles, have been omitted.

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AN ENDOCOCHLUS HAVING BINARY HELICOID THALLI OF LEFT-HANDED ROTATION

CHARLES DRECHSLER [1]

(WITH 6 FIGURES)

Several maizemeal-agar plate cultures which after having been overgrown with mycelium of *Pythium mamillatum* Meurs had been further planted on January 3, 1947, with small quantities of leaf mold kindly collected by Dr. E. B. Toole in an oak wood near Greensboro, North Carolina, in December, 1946, showed, on microscopical examination twenty-seven days later, sparse stands of erect beaked conidia arising in scattered areas from prostrate hyphae often arranged more or less radially. The general aspect of the conidial apparatus indicated clearly a species of *Endocochlus*. While the robust stature of the erect spores was rather strongly suggestive of my *E. gigas* (6: 368–371), their longer and accordingly much more conspicuous empty apical beaks made for an appearance alien to that species as well as to the smaller congeneric forms I have described under the binomials *E. asteroides* (5: 8–15) and *E. brachysporus* (6: 364–367). Closer examination, in which attention was given also to the vegetative and the sexual reproductive stages associated with the conidial apparatus, brought to light morphological peculiarities amply distinguishing the fungus here concerned as a new member of the genus.

Like the congeneric forms previously described, the new species subsists through parasitism on a relatively large terricolous *Amoeba*. Animals in the earlier stages of attack (FIG. 1, *A;* FIG. 2, *A;* FIG. 3, *A;* FIG. 4, *A*) were commonly found to measure from 100 to 130 μ in width when drawn into a rounded shape. They

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were enveloped individually by a firm pellicle that for the most part was cast into broadly undulating folds. Their hyaline, obscurely granular protoplasm surrounded a single prolate ellipsoidal nucleus (FIG. 1, *A, n;* FIG. 2, *A, n;* FIG. 3, *A, n;* FIG. 4, *A, n*), commonly 27 to 32 μ long and 18 to 21 μ wide, that contained at its periphery a large number of globose bodies about 1 μ in diameter. At the two poles of the nucleus these bodies, presumably consisting of chromatin material, were massed several layers deep, while in the equatorial region they were present usually in a single layer. Owing to the deeper accumulation of globose bodies at the poles the homogeneous central portion of the nucleus was often approximately equal in length and width. Many infected animals, as also many healthy individuals of the same species, contained one or more specimens of the testaceous rhizopod *Euglypha levis* (Ehrenb.) Perty (FIG. 1, *A, w;* FIG. 2, *A, w*), which, being abundant in the cultures, had apparently been ingested to serve as food material.

Because of close resemblances in nuclear organization, the protozoan here in question is held referable to *Amoeba papyracea* Penard (8: 201). While the measurements given by Penard for width of rounded individuals—minimum 176 μ, maximum 198 μ— are considerably in excess of those prevailing in my cultures, smaller dimensions would seem to be more or less normal in *Amoeba* populations developing on agar substrata. Previous to its appearance as the animal host of the new *Endocochlus* the protozoan had not been noted in the many agar plate cultures prepared from leaf mold from different sources over a period of more than ten years. Whether its failure to develop more often in plate cultures is due to limited distribution or to special requirements for multiplication, its absence in any material naturally precludes development of the zoöpagaceous form habitually subsisting on it.

Encounter between specimens of *Amoeba papyracea* and the fungus takes place much as in the several other species of *Endocochlus* made known earlier. When the *Amoeba* in its ordinary locomotion comes in contact with a conidium of the parasite the conidium remains affixed to the animal's pellicle, apparently through adhesion. From the adhering region the spore soon puts forth a bulbous protuberance, about 3 or 4 μ wide, which serves presumably as an ap-

pressorium since its rather thick wall usually shows later the yellowish coloration characteristic of adhesive structures in the Zoöpagaceae. The protuberance soon begins to elongate apically as a germ tube, in many instances at first pushing the pellicle inward to form a funnel-shaped depression. After the pellicle has been ruptured under increasing pressure the germ tube continues its growth some little distance into the protoplasmic interior. Elongation then ceases, and the narrowed tip of the germ tube abruptly begins expanding into a bulbous swelling (FIG. 1, *A, a*). Through progressive vacuolization the contents of the adhering conidium migrate gradually into the terminal swelling. When this migration is completed the externally adhering conidial envelope is wholly empty, as is also the germ tube on whose tip is then borne a prolate ellipsoidal or nuciform cell (FIG. 1, *A, b*) densely filled with protoplasm. Disturbances deriving from the movements of the animal readily suffice to detach the nuciform cell from its slender support. Following such disjunction the empty germ tube together with the conidial envelope is shed by the animal; while simultaneously the nuciform cell, or infective body (FIG. 1, *A, c*), immersed in its mobile protoplasmic ambient, begins autonomous parasitic development.

With the assimilation of nourishment from the host protoplasm, the infective body here sometimes grows out nearly as in the other known species of *Endocochlus,* by putting forth from its distal end a single prolongation that usually begins curving at an early stage (FIG. 1, *A, d, e;* FIG. 2, *A, a;* FIG. 3, *A, a-c*) to form a close helical coil (FIG. 1, *A, f;* FIG. 3, *A, d, e*), which, after describing one and one-half to two turns, may branch dichotomously (FIG. 3, *A, f, g*). Even where only a single thallodic coil is formed the present fungus shows for some time, if not to the end, a distinctive peculiarity in that the prolongation is, as a rule, at least in its proximal portion, conspicuously narrower than the infective body, so that this body stands out as a proximal swelling. In contrast, the infective bodies of congeneric species, and also of those species of *Cochlonema* having a similar manner of invasion, grow out distally at an increasing width and thus become merged indistinguishably with the resulting thallus.
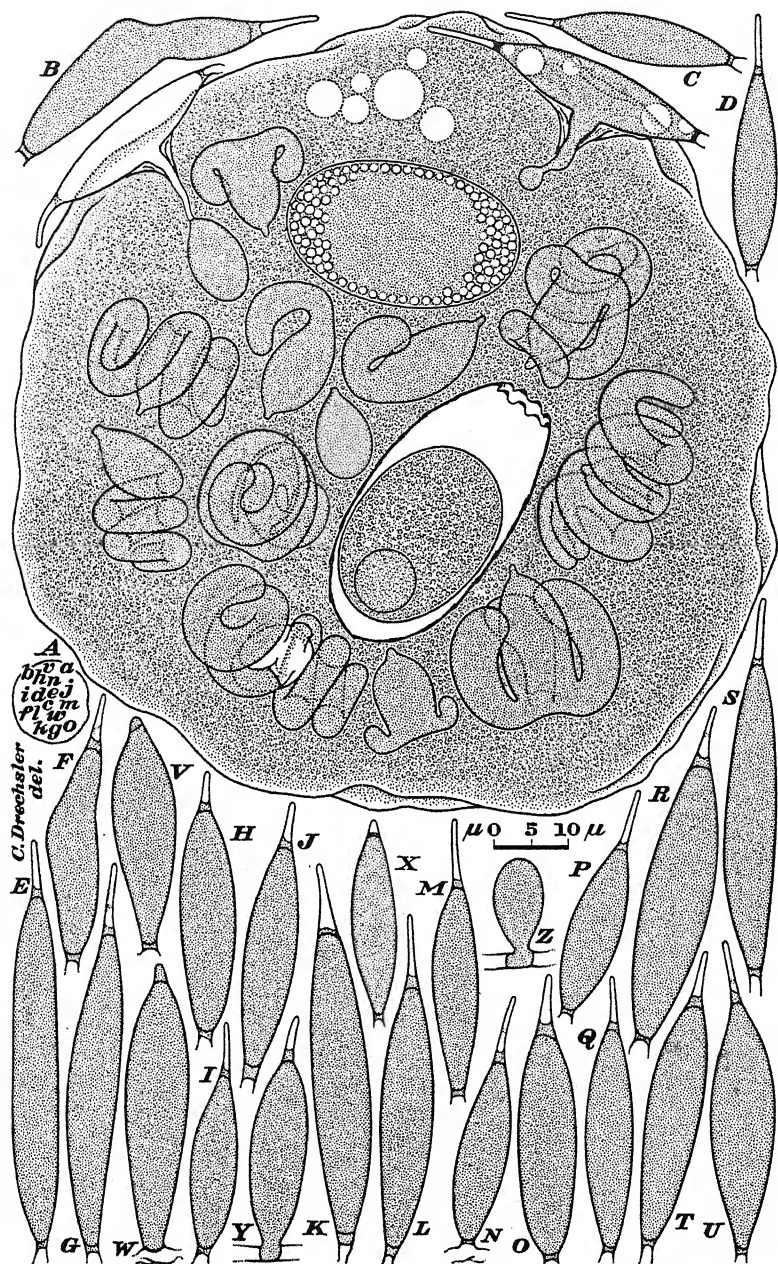
Fig. 1. *Endocochlus binarius.*

Although the production of a single thallodic coil is hardly infrequent in the present fungus, the much more usual manner of development here is by the extension of two thallodic coils. The two coils arise as small buds from opposite sides at the distal end of the infective body (FIG. 1, *A, g*). Through continued elongation, together usually with some distal widening and with constantly winding curvature (FIG. 1, *A, h*), they develop, as a rule, on opposite sides of the infective body, into helical coils which commonly remain unbranched until they have described one and one-half to two turns (FIG. 1, *A, i-l;* FIG. 2, *A, b-h;* FIG. 3, *A, h, i;* FIG. 4, *A, a, b*). Somewhat contrary to expectations that might be entertained, the coils are not oriented usually with their axes extending away directly from the sides of the infective body to which, respectively, they are attached, but instead are oriented with their axes perpendicular to the plane passing lengthwise through the middle of the infective cell and bisecting the basal attachments of both coils. The considerable measure of uniformity in positional relationships of the binary thallodic coils—a measure of uniformity seeming all the more remarkable because it is achieved despite constant disturbance from promiscuous movement of the protoplasmic ambient—is to be attributed to unusually consistent directive tendencies in growth that become manifest early in the elongation of the buds. After the initial abrupt curvature of the buds toward the proximal end of the infective body (FIG. 1, *A, g*), further curvature is such that if the infective body lies with its longitudinal axis in the plane of the microscopical field and with its proximal end directed toward the observer, the bud arising from the right side of the distal end will elongate with its advancing tip interposed between the observer and the infective body, while the bud from the left side of the distal end will elongate with its advancing tip passing underneath the infective body into partial concealment from the observer (FIG. 1, *A, h*). The three-dimensional curvature thus initiated is continued with further growth of the two buds; so that with the infective body oriented in the way just described, the bud arising on the right side of the distal end is soon found prolonged into a helical coil overlying the infective body and with its axis directed toward the observer in a line nearly parallel with his line of vision, while the bud arising from the left side of the distal end
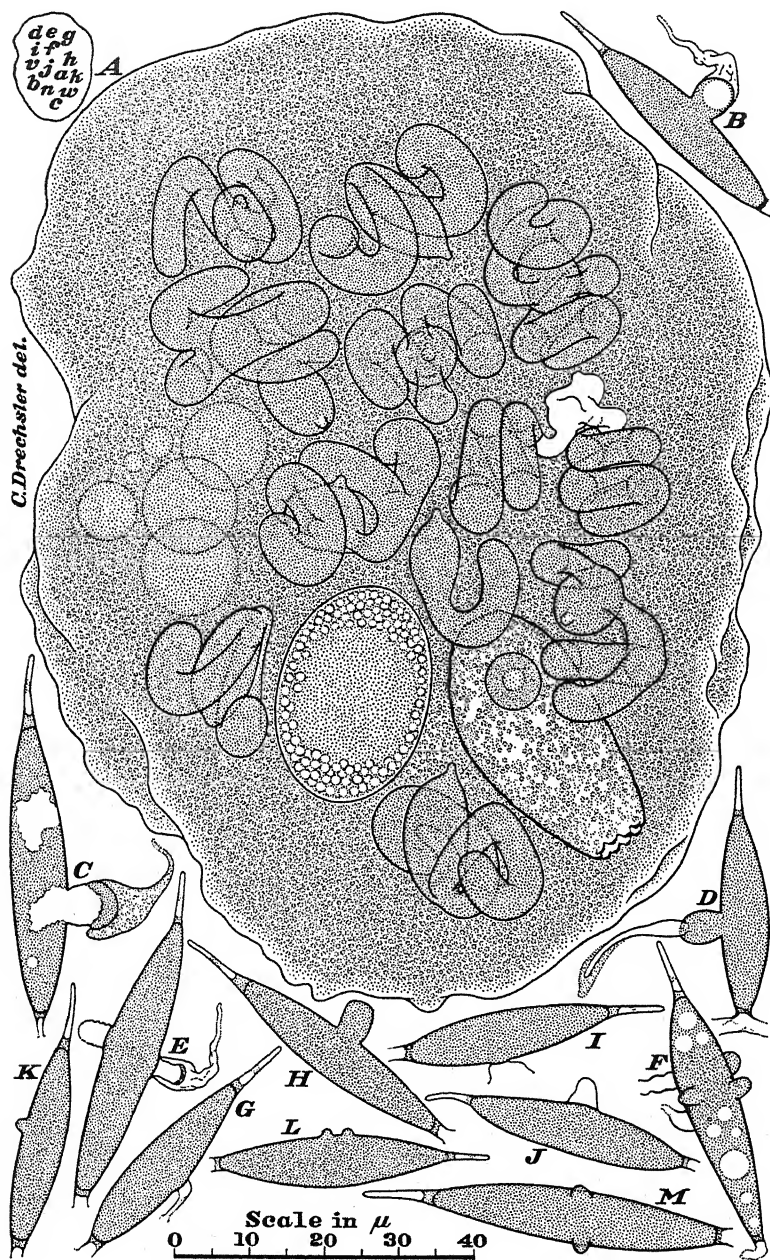
FIG. 2. *Endocochlus binarius.*

will be found extended into a similar coil underneath the infective body and with its axis directed vertically downward in a line likewise approximately parallel to the line of vision (FIG. 1, *A, l;* FIG. 3, *A, h;* FIG. 4, *A, b*). Curvature in similar relationship to the infective body and in the same direction of rotation prevails also where only one thallodic coil is produced, as well as in the occasional instances where three coils are formed (FIG. 1, *A, o*). Thus, regardless of variation in number, the coils consistently show rotation of the same direction as is found in the threads of a left-handed screw. To cite familiar examples from phanerogamic plants, the rotation shown here corresponds to that displayed in the twining stems of the common hop, *Humulus lupulus* L., and the black bindweed, *Polygonum convolvulus* L. Among fungi similar lefthanded rotation occurs as a distinctive feature in the helicoid ascospores of my *Cochliobolus heterostrophus* (**2, 4**), in the conidia of my *Harposporium helicoides* (**7**), in the coiled sporogenous tips of the sporophores of my *Helicocephalum oligosporum* (**3**), as well as in the aerial sporogenous branches and spore chains of some helicoid species of *Streptomyces* (= *Actinomyces*) as, for example, *S. lavendulae* (Waksm. & Curt.) Waksm. & Henr. (**1** : 150–151 ; **9** ; **10** : 944).

Since both of the two thallodic coils usually produced show rotation in the same direction they could well be regarded, especially during their earlier stages of growth, as making up a single binary helicoid structure modified midway between the ends by the presence of the protuberant infective cell. In many instances, however, the continuity of the combined structure is interrupted through evacuation of the infective body together usually with a proximal portion of the thallodic coils (FIG. 1, *A, k;* FIG. 2, *A, h;* FIG. 4, *A, a*) ; the larger, living portion of each coil then becoming delimited proximally by a retaining wall. The empty envelopes of infective bodies, after some time, often vanish completely from sight ; so that, eventually, many of the larger thallodic coils (FIG. 3, *A, j;* FIG. 4, *A, c*) come to appear little different, at the proximal end, from those of other species of *Endocochlus,* where the infective body, because of its broad elongation from the full width of its distal end, merges indistinguishably with the thallus.
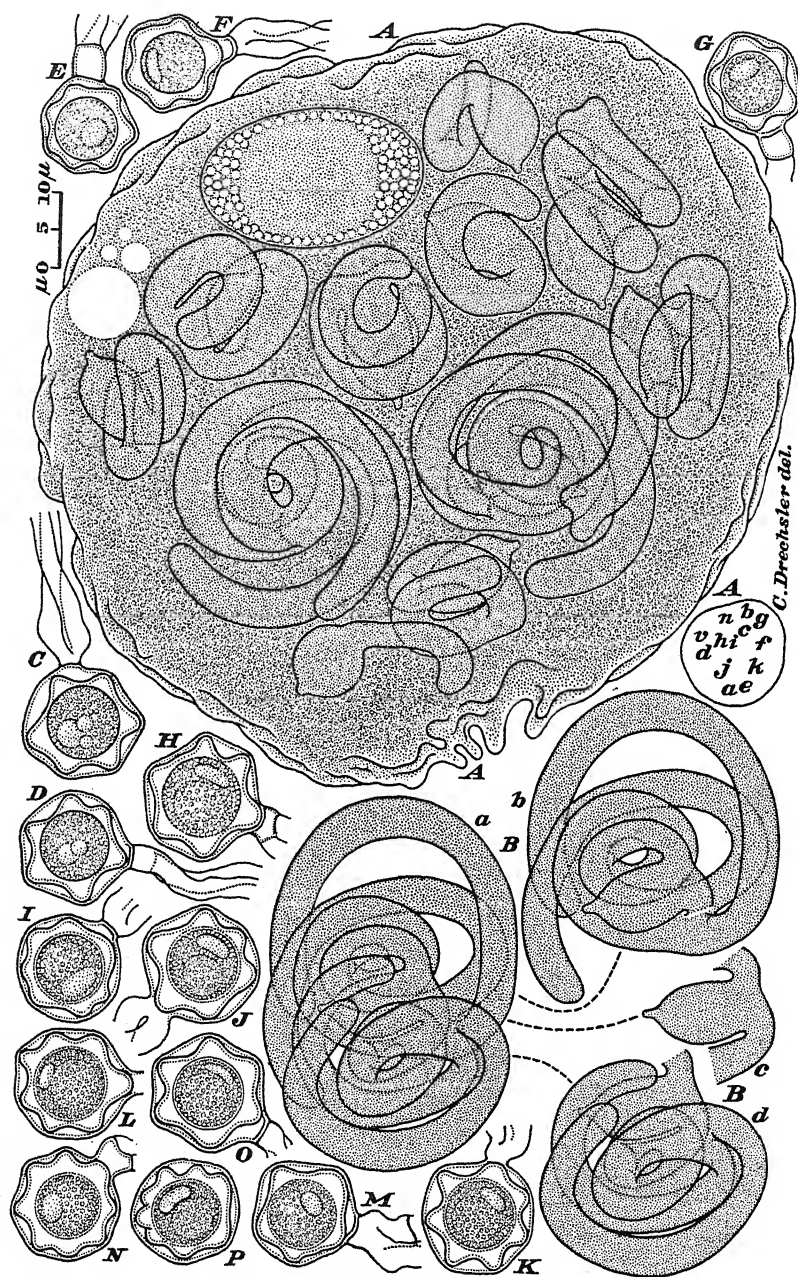
Fig. 3. *Endocochlus binarius.*

The larger thallodic coils are branched in varying measure, as many as three successive bifurcations often being recognizable (FIG. 4, *A, d*). Whether a coil is of solitary (FIG. 2, *A, i;* FIG. 3, *A, f, g*) or of twinned (FIG. 1, *A, m.* FIG. 3, *A, j, k; B, a-d.* FIG. 4, *A, c, d*) origin, its first dichotomy usually takes place after one and one-half to two turns have been formed, though thalli are not rare wherein much earlier bifurcation is clearly evident (FIG. 2, *A, j, k*). Following the first dichotomy, elongation of the branches is marked by diminished curvature, with the result that a thallodic coil whose first two turns may have an outer diameter equal to the length of the parent infective cell will after three successive dichotomies extend its eight branches rangily over an orbit three or four times wider (FIG. 4, *A, d*). Owing apparently to their rather open arrangement the longer terminal elements of well developed thalli are somewhat readily displaced from their original positions when they are jostled by other thalli in an animal under multiple attack. Accordingly in later stages of infection the larger thalli lose much of their geometrical symmetry as they are pressed together closer and closer. Before the protozoan host is incapacitated for further movement, primarily from advanced depletion of its protoplasm, the several thalli become confusedly intertangled in a dense clew (FIG. 5, *A, B*).

Although for a time after its disablement the infected specimen of *Amoeba papyracea* continues to operate its contractile vacuole (FIG. 5, *A, v; B, v*), further expropriation of its protoplasm and progressive degeneration of its nucleus (FIG. 5, *A, n; B, n*) eventually leads to the extinction of all signs of life. At this stage, or frequently a little earlier, the several thalli begin reproductive development. Where an infective cell is still present it often serves directly in putting forth one (FIG. 6, *A, a*) or more (FIG. 6, *B, a, b*) reproductive hyphae, while others are being extended from proximal positions on the associated coil (FIG. 6, *A, b*) or coils (FIG. 6, *B, c, d*). Naturally in those instances where the infective cell has been lost, all reproductive hyphae (FIG. 6, *C, a-h*) arise from the thallus, and in some instances all reproductive hyphae (FIG. 6, *D, a-g*) originate from the thallodic coils even where the infective body remains intact. The growth of the reproductive hyphae (FIG. 6, *E, a-d*) is accompanied by vacuolization and evacuation of
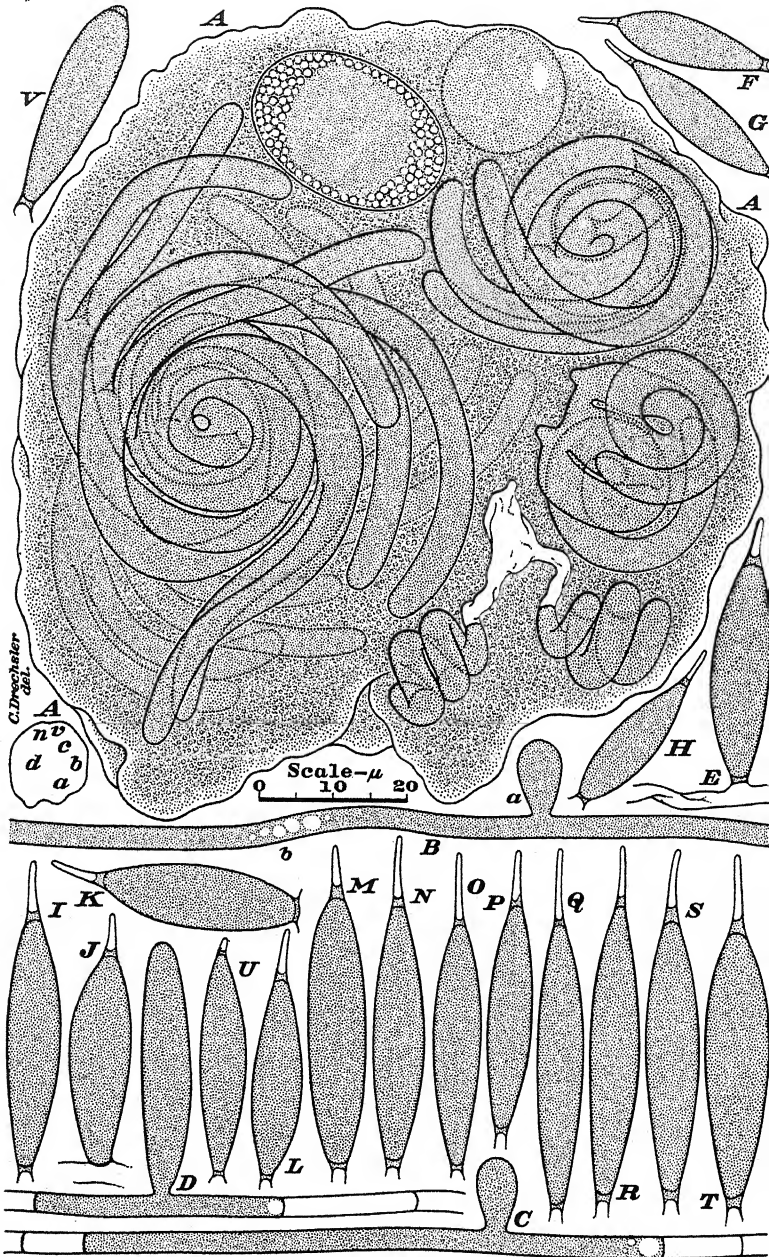
Fig. 4. *Endocochlus binarius.*

the thallus; the loss of contents usually becoming noticeable first in the distal parts of the coil (FIG. 6, *A*, *B*) and later in more proximal parts.   Retaining walls are laid down at intervals in the progressive withdrawal of protoplasm (FIG. 6, *E*), much as in the other species of *Endocochlus*, as well as in most species of *Cochlonema*.

The reproductive hyphae destined to serve in the development of conidia push their way to the animal's integument without establishing any definite positional relationships to one another.   On breaking through the pellicle they elongate radially from the dead animal until they extend procumbently over the surface of the culture for distances frequently of 2.5 to 3 mm.   They usually show only rather gently curving deviations from their generally straightforward courses, and only small departures from their usual width of about 3 μ.   After reaching their definitive length they soon put forth erect protuberances (FIG. 4, *B*, *a*; FIG. 5, *C*, *a-c*) often at intervals of 75 to 125 μ.   Thereupon the hyphal contents near the middle of each interval become noticeably vacuolate (FIG. 4, *B*, *b*).   As material is supplied for further growth of the protuberances the vacuolate portions are emptied and retaining walls are formed. Continued withdrawal of protoplasm soon leads to evacuation of an adjacent hyphal portion and deposition of another wall closer to the growing protuberance (FIG. 4, *C*.   FIG. 5, *D*, *a-c*; *E*).   With repetition of the process the living hyphal segment is further reduced (FIG. 4, *D*.   FIG. 5, *F*, *a*; *G*, *a*), and finally, as a rule, the remaining hyphal contents migrate into the erect outgrowth, which then is delimited a little above the base by a thickish septum (FIG. 4, *E*.   FIG. 5, *F*, *b*; *G*, *b*).   During the later stages of its growth, the erect body usually extends upward a narrow apical prolongation, which is soon delimited at the base by a rather thick wall or somewhat irregular plug-like partition.

Although the erect conidium thus formed resembles that of *Endocochlus gigas* in the fusoid shape of the living cell, it is of generally greater dimensions throughout (FIG. 1, *B-Z*.   FIG. 4, *F-V*.   FIG. 5, *H*, *a-z*; *I*, *a-z*; *J*, *a-z*).   It is distinguished more especially, however, by its narrow apical appendage, which as a rule is not only decidedly longer in actual measurement than the corresponding part in *E. gigas*, but is markedly longer also in comparison with the living cell; though in occasional specimens the appendage may be small
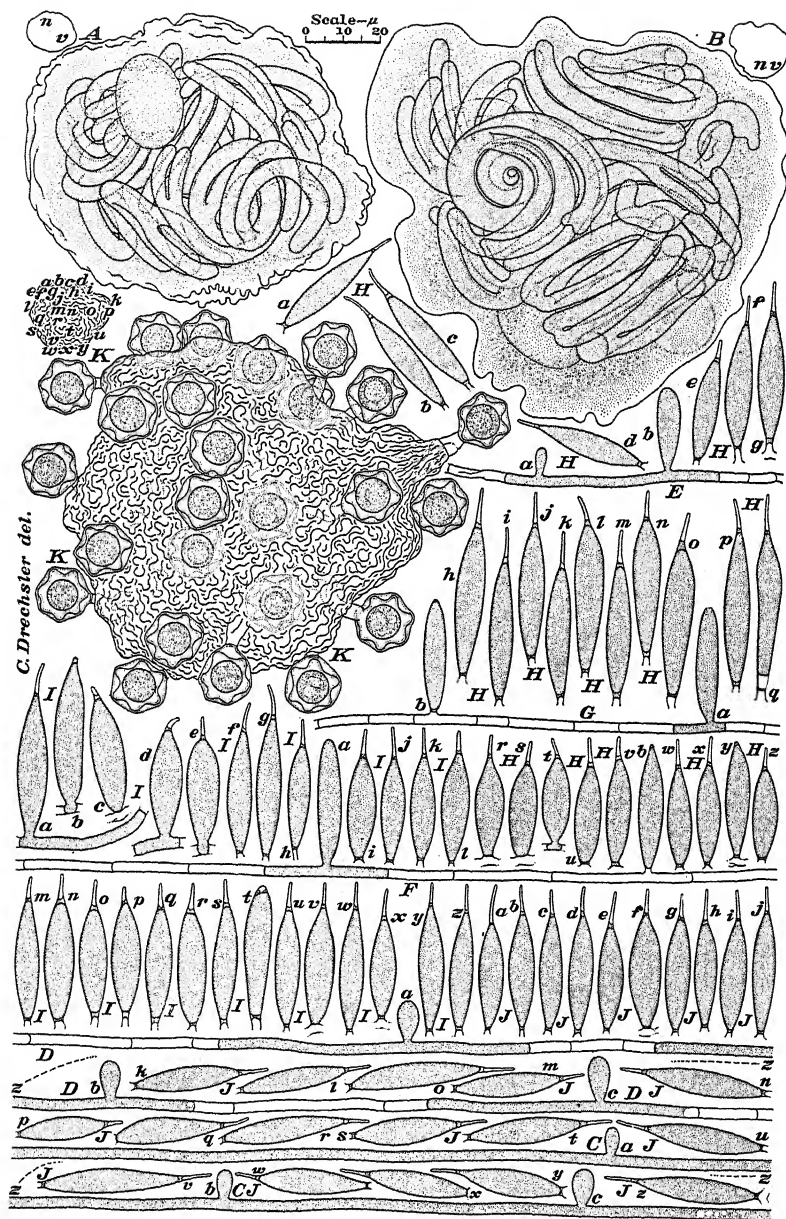
Fig. 5. *Endocochlus binarius.*

(FIG. 1, X; FIG. 4, U; FIG. 5, I, c, t), or may be present merely as a minute cap of solid wall material (FIG. 1, V, W.   FIG. 4, V.   FIG. 5, F, b; G, b; H, y; I, b), or in very imperfect specimens may be wholly absent (FIG. 1, Z).   When the mature conidium becomes detached, it does not usually separate from the empty hypha flush with the lower side of its basal wall but is abjointed commonly at a distance of 0.5 to 2 μ or more rarely as much as 3 μ, from this wall; so that the spore carries at its base a curious membranous prolongation, a little like an appendage.   This somewhat untidy manner of separation is not governed by chance, for the portion of hyphal membrane that accompanies the spore is always noticeably thicker than the hyphal envelope generally, and thus must have undergone earlier some special modification.   In some instances where the basal septum was formed flush with the parent hypha, the membranous prolongation may include a cylindrical portion of the hyphal envelope (FIG. 1, N. W.   FIG. 4, J.   FIG. 5, H, r, s, y; I, c, v, x; J, f, z).   Again, in occasional instances where because of incomplete transfer of protoplasmic contents the living cell of the detached spore includes a portion of the parent hypha (FIG. 1, Y, Z. FIG. 5, H, t; I, a, b, d, e), a short cylindrical portion of empty hyphal envelope is present at each of the two basal ends.

Germination has been observed taking place only in the conidia that have become affixed to the pellicle of a host animal.   Nevertheless, in cultures where the fungus has been active on a large scale for a few days, the conidia strewn about on the substratum usually include many individuals with lateral outgrowths and membranous attachments of various shapes (FIG. 2, B-M).   These modifications clearly derive from germinative development frustrated at different stages.   In resisting infection from individual conidia of the fungus, _Amoeba papyracea_ seems much more often successful than any other rhizopod I have hitherto seen undergoing attack by any zoöpagaceous parasite.   In several observed instances the animal protruded a pseudopodium at the place of attack, thereby eventually confining the germ hypha in a small narrow-necked pouch.   By persistent rotational movement of the animal the slender attachment was twisted off, and the pouch, consisting of a piece of pellicle and some protoplasm, was left behind adhering to the conidium (FIG. 2, C).   The host animal appeared to carry out
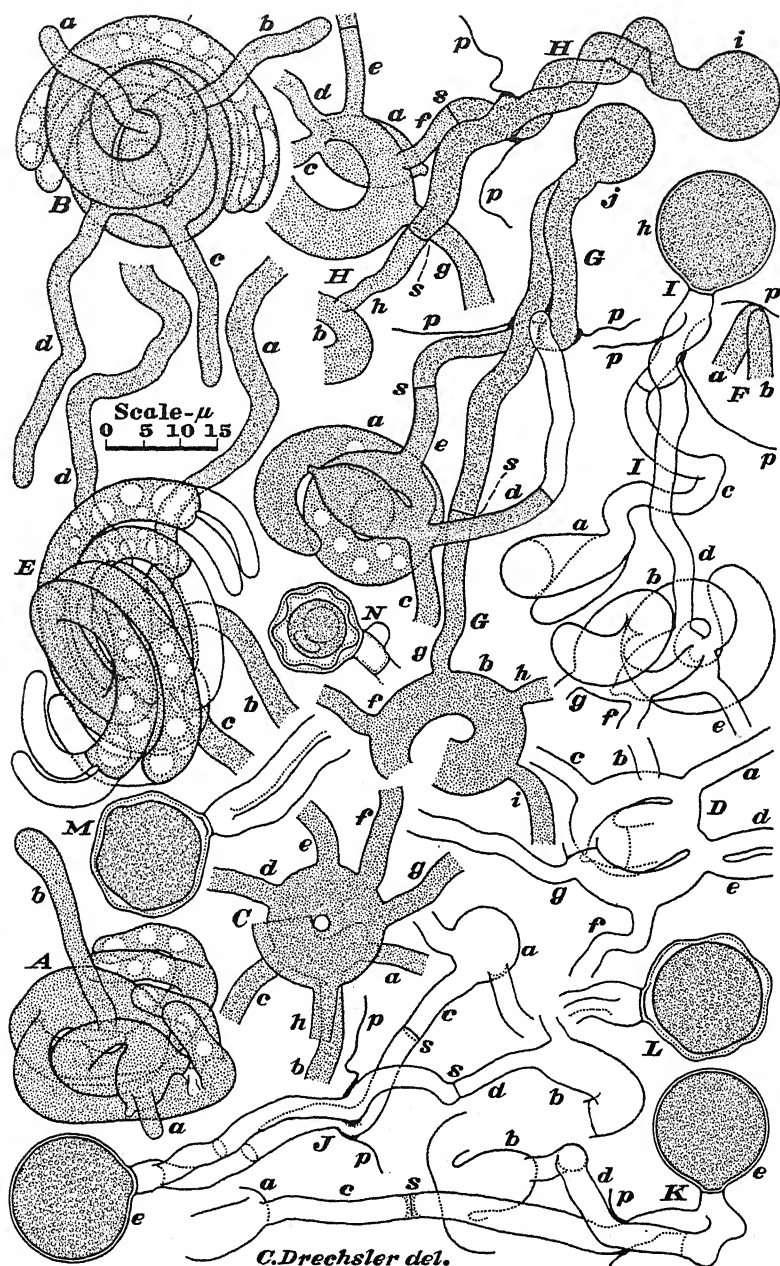
Fig. 6. *Endocochlus binarius.*

such defensive maneuvers repeatedly with different conidia, even when it was already infected with many growing thalli and thus was assuredly doomed. The conidia, on their part, were by no means rendered harmless by being sloughed off. If they had produced a long germ tube in their unsuccessful venture, the protoplasma in the tube was withdrawn backward into the spore envelope itself (FIG. 2, E-G), or into a short protruding stump (FIG. 2, B, D, H). If only appressoria had been formed, they were likewise evacuated when of large size (FIG. 2, J), but when relatively small (FIG. 2, K-M) were often left unchanged. Some conidia bore four or five frustrated germinative structures—appressoria and evacuated germ hyphae (FIG. 2, F)—thereby giving evidence of a corresponding number of unsuccessful attempts at infection.

The development of zygospores is initiated by reproductive hyphae of about the same width as those concerned in the formation of conidia. These hyphae make known their distinctive function by coming together in pairs inside the animal—the two members of a pair (FIG. 6, F, a, b) directing their growth in such wise as to bring their tips in contact with each other and at the same time with the inner surface of the animal's pellicle (FIG. 6, F, p). Pairing apparently never takes place between hyphae arising from the same vegetative thallus, single or binary; for in all instances where the difficulties of observation occasioned by the intertangling of fungus coils are not too troublesome, the two members of a pair are always traceable to separate thalli. Thus, in the reproductive apparatus shown in figure 6, G, where the two thalli a and b have each produced several reproductive hyphae, no pairing took place either between the three hyphae c-e arising from thallus a, or between the four hyphae f-i arising from thallus b, but the hypha e from thallus a was found paired with the hypha g from thallus b. Likewise in figure 6, H, showing portions of two thalli, a and b, no pairing could be made out between any of the five reproductive hyphae c-g arising from thallus a, but one of them, f, had paired with a hypha, h, coming from thallus b. Again in the reproductive apparatus shown in figure 6, I, the two empty thalli a and b are seen to have contributed the paired hyphae c and d, respectively, although the three additional hyphae e-g arising from thallus b have not paired with one another. Further, in the unit of sexual

apparatus shown in figure 6, *J,* the empty thalli *a* and *b* have contributed the paired zygophoric hyphae *c* and *d,* respectively; while similarly in the unit of sexual apparatus illustrated in figure 6, *K,* the empty thallus *a* and the empty infective body *b,* separate from it, have given off the paired zygophoric hyphae *c* and *d,* respectively.

The paired zygophoric hyphae, on making apical contact with each other and also with the host animal's pellicle (FIG. 6, *G-K: p*), secrete at their tips some yellow adhesive material. Thus anchored securely to the pellicle, they break jointly through this envelope, and then elongate externally side by side for distances varying commonly from 5 to 35 μ before fusing at the tip. As a rule, and more especially where elongation takes place under the surface of a culture, the hyphae follow rather straightforward courses (FIG. 6, *G*). Where external elongation takes place on the surface of a culture the zygophoric hyphae in some instances wind spirally about one another, their direction of rotation then being indiscriminately either right-handed (FIG. 6, *H*) or left-handed, rather than consistently left-handed like the rotation in the thallodic coils.

When their elongation ceases the paired hyphae fuse apically and begin to bud forth a globose body (FIG. 6, *G, j; H, i*) directly at the place of union, or terminally on a short stout prolongation usually not more than 2 μ in length. Meanwhile a cross-wall (FIG. 6, *G, s; H, s; J, s; K, s*) proximally delimiting a gametangium makes its appearance in each of the paired hyphae and usually in a position well within the host pellicle. The globose body increases gradually in size as it receives protoplasmic material from the gametangia. When the gametangia have given all their contents, it usually has reached a definitive diameter of approximately 15 μ (FIG. 6, *I, h; J, e; K, e*) and then apparently becomes delimited at or near the base by a retaining wall. The protoplast of the subspherical zygosporangium thus set off soon shrinks away from the enveloping wall, which thereupon usually collapses slightly to present a somewhat wavy contour (FIG. 6, *L*). Later the protoplast takes on an angular shape (FIG. 6, *M*), preparatory evidently to the formation of a yellowish zygospore wall with a pronouncedly warty or stellate outline. Within this wall the originally granular contents round up into a spherical living cell (FIG. 3, *C-P;* FIG. 6, *N*). At maturity this cell seems to be constituted, as a rule, of an outer granu-

lar layer surrounding a more nearly homogeneous reserve globule and having imbedded in it a somewhat flattened refringent body. The internal organization of the resting zygospore here as in the several congeneric forms and in most other members of the Zoöpagaceae would seem to correspond to the unitary organization familiar in the resting oospores of many oomycetes, including most species of *Pythium* and all species of *Aphanomyces*.

As the individual zygospores are produced close to the host pellicle in numbers ranging usually from 10 to 25 (FIG. 5, *K, a-y*)—the greatest number noted was 37—and are of durable character, they arrest attention by their clustered arrangement for months after all vestiges of their protozoan hosts have vanished from sight. In some plate cultures many were observed being invaded and destroyed by a hyphomycete that developed mainly through parasitism on the oospores of *Pythium mamillatum*. Though a mycelial connection could not be traced the hyphomycete in question was most probably *Trinacrium subtile* Fres., since the distinctive conidia of that oospore parasite were present in some abundance. The same hyphomycete also parasitized many specimens of the testaceous rhizopod *Arcella vulgaris* Ehrenb. that were distributed on the surface of the agar, and further invaded numerous globose cysts which had been produced in the cultures by a member of the Myxobacteriaceae.

A term having reference to its predominantly binary vegetative development is deemed suitable as a specific name for the new fungus.

Endocochlus binarius sp. nov.

Corpus intromissum per tubulum germinationis nuciforme, plerumque 12–20 μ longum, 6–10 μ crassum, basi acutulum, apice primo rotundum, mox ex apice vulgo 2 (interdum 1 et rarius 3) hyphas assumentes pullulans, postea aliquando 1 vel 2 hyphas genitabiles (hyphas conidiiferas vel zygosporiferas) uspiam emittens; hyphis assumentibus incoloratis, prope basim 2–4.5 μ crassis, sursum leniter quandoque usque 8 μ latescentibus, usque 200 μ longis, in spiram semel vel bis vel ultra quam ter ad modum caulis Humuli lupuli cochleatim convolutis, nunc simplicibus nunc semel usque ter repetite bifurcis, animal debilitato vel moribundo prope originem ex latere convexo 1–8 hyphas genitabiles emittentibus. Hyphae conidiiferae per pelliculam animalis solitatim erumpentes, extra animal procumbentes et paene rectae, simplices vel parve ramosae, incoloratae, saepe 2.5–3 mm. longae, plerumque 2.5–3.3 μ crassae, primo continuae et protoplasmatis repletae, post auctum conidiorum

inanes et septatae; conidiis inter se saepius 75–125 μ distantibus, incoloratis, erectis, post disjunctionem deorsum subter septo inferiore membrana circulari inani 1–3 μ longa praeditis, vulgo in cellula viventi et appendice vacua terminali consistentibus; cellula viventi ellipsoidea vel fusiformi, 20–45 μ longa, 5.7–9 μ crassa; appendice vacua 2–10 μ longa, deorsum plerumque 1–1.5 μ crassa, apice 0.6–0.8 μ crassa. Hyphae zygosporiferae 2–4.5 μ crassae, ambae ex aliis hyphis assumentibus oriundae, apice ad pelliculam animalis communiter inhaerentes, mox ex pellicula communiter erumpentes, communiter 5–35 μ in longitudine interdum spiraliter interdum recto crescentes, denique gametangiis terminalibus saepius 20–50 μ longis seclusis in apice conjungentes et zygosporangium ex junctione gignentes; zygosporangio globoso, plerumque 12–17 μ in diametro, membrana ejus in maturitate circum zygosporam laxe collapsa; zygospora flavida globosa, 11–15 μ crassa, membrana in maturitate valde verrucosa, cellulam viventem sphaeralem 8–10.5 μ crassam circumdante.

Amoebam papyraceam enecans habitat in foliis Quercus putrescentibus prope Greensboro, North Carolina.

Infective body formed on intruded germ tube usually elongated ellipsoidal, minutely protruding at the proximal end, rounded at the distal end, usually 12–20 μ long and 6–10 μ wide, from opposite positions on the distal end soon putting forth 2 (sometimes 1 and more rarely 3) assimilative hyphae, and later, on disablement or death of animal host, sometimes extending from indeterminate positions 1–2 reproductive (*i.e.*, conidiiferous or zygophoric) hyphae. Assimilative hyphae colorless, up to 200 μ long, near their attachment usually 2–4.5 μ wide, usually widening rather gradually to attain more distally sometimes a diameter of 8 μ, convolved in a helicoid spiral of 1–3.5 turns with left-handed rotation, simple or once-dichotomous or successively bifurcated 2–3 times; after disablement or death of host animal giving rise proximally from the convex side to reproductive hyphae in numbers ranging from 1 to 8. Conidiiferous hyphae erupting individually from the animal's pellicle to elongate procumbently in rather straightforward courses for distances frequently of 2.5–3 mm. at a width between 2.5 and 3.3 μ, simple or sparingly branched, at first continuous and filled with protoplasm, but becoming empty and septate in producing erect conidia at intervals frequently of 75–125 μ. Conidia colorless, consisting individually of an elongated ellipsoidal or spindle-shaped living cell, 20–45 μ long and 5.7–9 μ wide, surmounted usually by an empty tapering appendage 2–10 μ long, 1–1.5 μ wide proximally, and tapering to a width of 0.6–0.8 μ near the tip; the individual spore usually becoming detached a short distance below its basal septum, and thus bearing proximally an empty collar-like membranous prolongation 1–3 μ long. Zygophoric hyphae 2–4.5 μ wide, each pair arising from separate assimilative structures, the

two usually making close contact at their apices before breaking jointly through the host pellicle to elongate together outside 5–35 μ usually in a straightforward course but sometimes winding spirally about one another, then (terminal gametangia often 20–50 μ long having been delimited) fusing at the tip and budding forth a zygosporangium from the place of union. Zygosporangium globose, usually 12–17 μ in diameter, its wall at maturity collapsing loosely about the zygospore. Zygospore subspherical, 11–15 μ in diameter, slightly yellowish, its wall at maturity presenting a somewhat stellate profile around a spherical protoplast 8–10.5 μ in diameter.

Destroying *Amoeba papyracea* it occurs in decaying Quercus leaves near Greensboro, North Carolina.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND

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EXPLANATION OF FIGURES

FIG. 1. *Endocochlus binarius,* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A,* Specimen of *Amoeba papyracea* in active condition though under multiple attack by the fungus, with

the fungus in various stages of development: *a*, adhering conidium with a funnel-shaped germinative outgrowth that has penetrated the animal's pellicle and is forming terminally an infective body in the host protoplasm; *b*, adhering conidial envelope that has been emptied by movement of its contents through the tapering germ tube into the terminal infective body, which now is fully formed and delimited at its base by a retaining wall; *c*, newly detached infective body; *d, e*, two infective bodies, each of which has begun to grow out distally into a single thallodic coil; *f*, an infective body that has grown out distally into a single thallodic coil of two turns; *g*, infective body that is budding out in opposite positions at its distal end, to begin the development of two thallodic coils; *h*, similar infective body whose two distal outgrowths are somewhat longer and thus show in the orientation of their expanded tips the left-handed rotation characteristic of the vegetative stage of the fungus; *i*, infective body with two regularly convoluted thallodic coils, each coil consisting of one and one-half turns; *j*, infective body with two less regularly convoluted thallodic coils, each consisting of approximately one and one-fourth turns; *k*, empty infective body that has produced two regularly convoluted thallodic coils, each of approximately two turns and each delimited proximally by a retaining wall; *l*, infective body with two regularly convoluted thallodic coils shown endwise, each coil consisting of about one and one-half turns; *m*, infective body with two regularly convoluted thallodic coils, each of which is bifurcated about one and one-half turns from its origin; *n*, nucleus of animal host; *o*, large infective body that has put forth three thallodic outgrowths, each disposed in a left-handed coil of about one-half turn; *v*, contractile vacuole of animal host; *w*, encysted specimen of *Euglypha levis* ingested by animal host. *B–U*, Conidia of usual make-up, showing ordinary variations in size and shape, and in length of empty beak. *V, W*, Conidia with short apical beaks that apparently are not empty. *X*, Conidium with small empty apical beak. *Y*, Conidium including at the base a portion of the parent hypha. *Z*, Conidium lacking an apical beak, and at the base including a portion of the parent hypha.

FIG. 2. *Endocochlus binarius*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Specimen of *Amoeba papyracca* still actively motile though under multiple attack by the fungus: *a*, infective body that has grown out distally to form a young thallodic coil of one-half turn; *b*, infective body with two regularly convoluted thallodic coils, each consisting of approximately a three-fourth turn; *c*, infective body with two somewhat irregularly convoluted thallodic coils, each comprising approximately one turn; *d*, infective body (viewed from the proximal end) with two thallodic coils, each composed of one turn; *e*, infective body with two regularly convoluted thallodic coils, each comprising slightly more than one turn; *f*, infective body (viewed from the distal end) bearing two regularly convoluted thallodic coils, each of slightly more than one turn; *g*, infective body (likewise viewed from distal end) bearing two regularly convoluted thallodic coils, each comprising about one and one-half turns; *h*, empty collapsed membranous envelope of an infective body, to which are attached two regularly convoluted thallodic coils, each delimited proximally by a retaining wall and comprising two turns; *i*, infective body that has grown out into a single thallodic coil, which bifurcated one and three-fourth turns from its

origin; *j, k,* infective bodies (in oblique view), each with two thallodic coils, one of them being simple, the other one being bifurcated about one-half turn from its origin; *n,* nucleus of animal host; *v,* contractile vacuole of host; *w,* ingested specimen of *Euglypha levis.* *B–M,* Conidia with various lateral modifications ranging from 1 to 5 in number, each resulting from frustrated infective germination; in *C* the broad adhesive appressorium has attached to it a portion of pellicle and protoplasm torn from an animal in its successful escape; in *D* the distally evacuated germ tube is shown encased in a remnant of protoplasm from an animal that escaped infection after deep penetration by the fungus.

Fig. 3. *Endocochlus binarius,* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A,* Specimen of *Amoeba papyracea* still in motile condition though under multiple attack by the fungus: *a,* infective body that has grown out distally into a single thallodic prolongation, which began coiling later than is usual; *b, c,* infective bodies that have each grown out into a single thallodic coil of a three-fourth turn; *d, e,* infective bodies, each of which has grown out into an irregularly convolved thallodic coil of one and one-half turns; *f, g,* infective bodies, each of which has grown out into a single thallodic coil that is bifurcating after describing one and three-fourth turns; *h,* infective body with two thallodic coils, each comprising a single turn; *i,* infective body with two thallodic coils of a single turn, the coil attached on the left showing departure from the usual in having taken a course on the same side of the infective body as the coil attached on the right; *j,* single coiled bifurcate thallus comprising two and one-half successive turns, which is not attached to any visible infective body; *k,* infective body bearing two thallodic coils, only one of which is shown with its two and one-half successive turns and its two bifurcations; *n,* nucleus of animal host; *v,* contractile vacuole of animal. *B,* Infective body with two thallodic coils, each of them once bifurcate and each consisting of two and one-half successive turns: *a,* the complete assimilative structure, with all parts in place; *b,* the infective body with the one thallodic coil underneath it; *c,* the infective body with a proximal portion of each thallodic coil; *d,* the infective body with the one thallodic coil overlying it. *C, D,* Portions of empty paired zygophoric hyphae whereon are borne globose zygosporangia, each containing a zygospore somewhat immature in the organization of its protoplast. *E–P,* Zygosporangia, each containing a zygospore having a prominently warty envelope and a subspherical protoplast of mature structure.

Fig. 4. *Endocochlus binarius,* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A,* Specimen of *Amoeba papyracea* still in active condition though under multiple attack by the fungus: *a,* empty membranous envelope of infective body bearing two regularly convolved thalli, each comprising two and one-half turns and each delimited proximally by a retaining wall; *b,* infective body bearing two regularly convolved unbranched thallodic coils, each consisting of one and three-fourth turns; *c,* thallodic coil, comprising two and one-half successive turns and showing two bifurcations, which no longer is attached to any visible infective body; *d,* well developed thallodic coil, convolved in three to three and one-half successive turns and showing three successive bifurcations, which lies over its companion whereof only the eight terminations directly exposed

to view are shown, their contours being drawn with stippled lines; *n*, nucleus of animal host; *v*, contractile vacuole of animal. *B*, Portion of a continuous prostrate conidiiferous hypha showing a conidium, *a*, in early stage of development, and a cluster of vacuoles, *b*, resulting from initial loss of hyphal contents. *C*, Portion of prostrate conidiiferous hypha showing a young conidium, and several partitions formed on progressive evacuation of hyphal parts. *D*, Portion of prostrate conidiiferous hypha showing a conidium in more advanced stage of development. *E*, Portion of empty prostrate hypha with a fully developed conidium. *F–U*, Fully developed conidia showing usual variations in size and shape of both the living cell and the empty apical beak. *V*, Conidium in which the apical beak is reduced to a thickened cap.

Fig. 5. *Endocochlus binarius*, drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A, B*, Two specimens of *Amoeba papyracea* no longer capable of locomotion owing to advanced infection of each by approximately ten thalli variously intertangled and massed in a confused clew; the two animals, despite marked degeneration of their nuclei, *n*, and reduction of their protoplasm to about one-tenth of the normal quantity, still showing life in continued operation of their contractile vacuoles, *v*. *C*, Portion of continuous conidiiferous hypha showing three conidia, *a–c*, in early stage of development; owing to lack of space the hyphal portion is shown in two parts connecting at the point *z*. *D*, Portion of conidiiferous hypha with empty intervals that were evacuated and partitioned owing to progressive movement of contents into the three growing conidia *a–c*; the hyphal portion being shown, from lack of space, in two parts connecting at the point *z*. *E*, Short portion of conidiiferous hypha on which two conidia, *a* and *b*, are being formed. *F, G*, Largely evacuated portions of conidiiferous hypha, each showing a conidium, *a*, in advanced stage of development, and a fully developed conidium, *b*. *H–J*, Random assortment of conidia, *a–z*, showing variations in size and shape of the living cell and of the apical beak, as well as in the length of the basal membranous prolongation; a few examples (*H, t; I, a, b, d, e*) showing variable extension of protoplasm into the parent hypha. *K*, Wrinkled collapsed pellicle of a large specimen of *Amoeba papyracea* surrounded by twenty-five zygosporangia of the parasite, *a–y*, each containing a prominently warty zygospore with a spherical protoplast in mature resting condition.

Fig. 6. *Endocochlus binarius*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Infective body with a twice bifurcated thallodic coil of two successive turns; one reproductive hypha, *a*, is growing out directly from the infective body, and another, *b*, from the proximal portion of the coil. *B*, Infective body with two thallodic coils, each comprising one and three-fourth turns and bifurcating once; two reproductive hyphae, *a* and *b*, are growing out from the infective body, while two others, *c* and *d*, are being extended from the basal portions of the coils. *C*, Proximal portion of a thallodic coil from which eight reproductive hyphae, *a–h*, have been extended; the infective body from which the coil originated is no longer visible. *D*, Empty membranous envelope of an infective body and of proximal portions of two thallodic coils; three empty reproductive hyphae, *a–c*, are shown arising from one coil, and four similar hyphae, *d–g*,

are shown arising from the other. *E,* Branched helicoid thallus with six terminal elements, each of which is partly or wholly emptied and provided with a retaining wall; below these walls the contents show pronounced vacuolization; four reproductive hyphae, *a–d,* are given off from the proximal turn. *F,* Tips of two zygophoric hyphae, *a* and *b,* making contact on the inner side of the host pellicle, *p. G,* Two helicoid thalli, *a* and *b,* the former having put forth three reproductive hyphae, *c–e,* the latter having put forth four such hyphae, *f–i;* after the hyphae *e* and *g* had paired by tip-to-tip contact on the inner surface of the host pellicle, *p,* they grew through the pellicle side by side for a distance of 20 μ and then fused apically to begin development of the partly grown zygosporangium, *j;* a septum, *s,* had in the meantime been formed in each hypha to delimit the paired gametangia; the hypha *d* is present as a frustrated supernumerary zygophore. *H,* Two helicoid thalli, *a* and *b,* that have put forth the reproductive hyphae *c–h;* the hypha *f* (from thallus *a*) having paired with hypha *h* (from thallus *b*) by apical contact on the inner surface of the host pellicle, *p,* the two broke jointly through the pellicle and elongated outside for a distance of 25 μ while winding about one another before they fused apically to give rise to the globose zygosporangium *i;* a septum, *s,* having meanwhile been formed in each zygophoric hypha to delimit the paired gametangia. *I,* Two empty helicoid thalli, *a* and *b,* from which were put forth the reproductive hyphae *c–g;* after the hyphae *c* and *d* (originating from thalli *a* and *b,* respectively) had paired by making contact on the inner surface of the host pellicle, *p,* the two erupted jointly to elongate externally for a distance of 5 μ, and then fused apically to form the zygosporangium *h,* now fully grown and delimited at the base. *J,* Parts of two empty thalli, *a* and *b,* from which were extended the zygophoric hyphae *c* and *d,* respectively; the two hyphae, after becoming paired by adhering together apically on the inner side of the host pellicle, *p,* having erupted and grown externally side by side for a distance of 25 μ, then having fused apically to form the zygosporangium *e,* now fully grown and delimited at the base; *s,* septa delimiting the paired gametangia. *K,* Portion of empty thallus, *a,* and an empty infective body, *b,* from which were extended the zygophoric hyphae *c* and *d,* respectively; these hyphae, after becoming paired through mutual adhesion on the inner side of the host pellicle, *p,* having erupted together and grown externally side by side for a distance of 15 μ, then having fused apically to form the zygosporangium, now fully grown and delimited at the base; *s,* septum delimiting the gametangium supplied by hypha *c. L, M,* Two zygosporangia whose protoplasts have shrunken away from the slightly collapsed envelopes to form young zygospores. *N,* Slightly collapsed zygosporangial envelope surrounding a mature zygospore with boldly verrucose wall and subspherical protoplast.

A SECOND CONTRIBUTION TO THE KNOWLEDGE OF THE USTILAGI-NALES OF CHINA [1]

Lee Ling

(WITH 2 FIGURES)

The present contribution deals primarily with the Ustilaginales collected in Taiwan (Formosa) of South China. The materials studied were obtained from the herbarium of the Taiwan Agricultural Research Institute, the herbarium of the National University of Taiwan, and the personal collection of Y. Hashioka.

The first record of Ustilaginales from Taiwan was made by P. Hennings (1). He recorded three species, among which *Ustilago digitariae* (Kze.) Rabh. has not been reported since under that name although it is probable that it is the same species now known as *Sorosporium formosanum* Saw. Later both Sawada and Ito studied this group of fungi from this region. All the species recorded by them have been examined by the writer and several of the species described by them have been reduced to synonymy, with the result that the total number of species previously reported from the island has been reduced to forty-six.

Since several species described by Sawada after 1935 were not provided with Latin diagnoses, these are supplied here to legitimatize their publication. These diagnoses, however, are chiefly based upon the observations of the writer rather than upon translations from the original.

TILLETIACEAE

1. **Tilletia alopecuri** (Saw.) Ling, comb. nov. (FIG. 1, a; and 2, a)
   Entyloma alopecuri Saw., Dept. Agr. Gov't Res. Inst. Formosa Rept. 2: 86. 1922.

Sori in the ovaries, infecting most of the spikelets, inconspicuous, hidden completely by the enveloping glumes and each covered

[1] The first of this series was published in Mycological Papers, Kew Mycological Institute, No. 11. 1945.
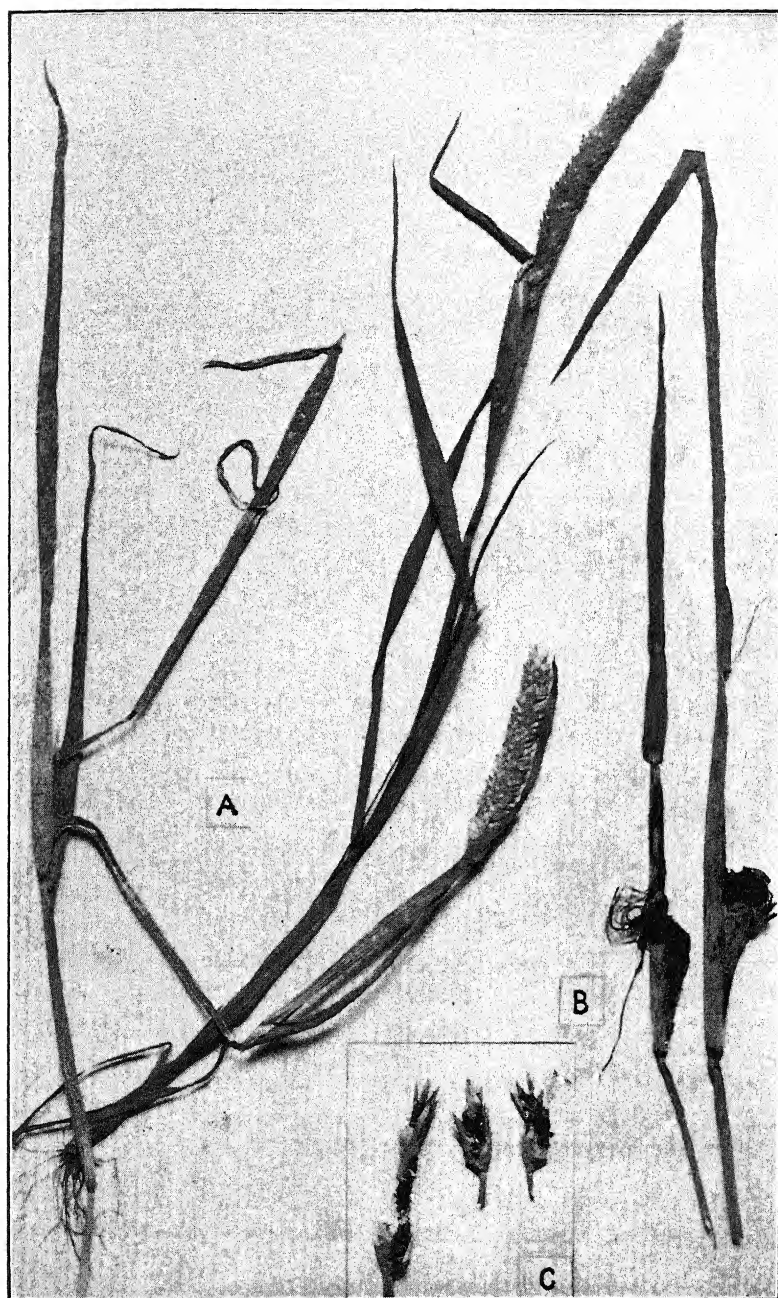
Fig. 1. Ustilaginales of China.

by a thin membrane of host tissue, broadly elliptical with a tapering apex, approximately 1 mm. long; also in leaves, leaf sheaths, and internodes of culms, forming short or elongate, yellow striae, later becoming dark brown, the infected parts of culms hypertrophied, often causing curvature and bulging of the culms; chlamydospores light yellow, spherical to subspherical, enclosed in a hyaline envelope, 17–25.5 μ diam., with a mean of 22.2 μ, covered with prominent spiny scales measuring 2–3 μ high by 2.5–3.5 μ wide at base, germination by a short promycelium with an apical whorl of 16–18 primary sporidia; sterile cells almost hyaline, 12–16.5 μ, walls 1.5 μ thick; conidia formed on surface of infected vegetative tissues of the host, hyaline, smooth, 14–35 × 5–8 μ.

On *Alopecurus geniculatus* L., Taipeh, coll. K. Sawada (cotype), Jan. 19, 1914.

This fungus infects the ovaries as well as the vegetative organs of the host. Sawada, in his original description, apparently overlooked the presence of infection in the ovaries and was probably also impressed by the occurrence of conidia in nature, which is very unusual for *Tilletia*. Those facts led him to place it in the genus *Entyloma*.

In Arkansas, U. S., a similar fungus, described as *Tilletia youngii* Clint. & Zundel, was found on *Alopecurus carolinianus*. The type of that species agrees well with *T. alopecuri* in both the symptoms caused and the general appearance of spores, though in its original description the presence of sori on leaves and leaf sheaths was neglected. *T. youngii,* however, has larger spores measuring 19.5–27 μ diam. with an average of 25.1 μ and coarser spiny scales which are 2.5–4.7 μ wide at base.

2. NEOVOSSIA HORRIDA (Takah.) Padw. & Azmatullah Khan, Mycol. Papers **10**: 2. 1944.

On *Oryza sativa* L., Taipeh, coll. K. Sawada, Oct. 28, 1926.

3. ENTYLOMA AUSTRALE Speg., Anal. Soc. Ci. Argent. **10**: 5. 1880.

On *Physalis angulata* L., Yüli, Hualienhsien, coll. K. Sawada, May 13, 1909.

4. ENTYLOMA BIDENTIS P. Henn., Pflanzenw. Ost-Afrikas C. **5**: 49. 1895.

On *Bidens pilosa* L., Taipeh, coll. K. Sawada, June 5, 1942.

5. **Entyloma eleocharidis** (Saw.) Ling, comb. nov. (Fig. 2, c)
   Ustilago eleocharidis Saw., Taiwan Agr. Res. Inst. Rept. **85** : 39.
   1943.

Soris in culmorum partibus superioribus, circularibus vel irregularibus, plumbeo-nigris, 0.1–1 mm. longis; sporis in columnis paralellibus catenatis inter epidermides evolutis, globosis usque subglobosis, saepe angularibus, 9–12 × 7–10.5 μ, episporio tenui, dilute olivaceo-brunneo, levi.

In culmis *Eleocharidis dulcis* Trin.

Sori on the upper parts of the culms, circular or irregular, lead black, 0.1–1 mm. long; spores embedded permanently in the host tissues between the upper and lower epidermis, arranged in parallel columns, globose to subglobose, often angular, 9–12 × 7–10.5 μ, epispore thin, smooth, light olivaceous brown.

On *Eleocharis dulcis* Trin., Taipeh, coll. K. Sawada (*type*), Nov. 16, 1934.

Although the taxonomic position of this species cannot be ascertained without knowledge of its spore germination, the appearance of sori and spores indicates that it is more likely to be an *Entyloma* than a *Ustilago*. A similar fungus, *Entyloma parvum* Davis, occurs in North America on the same host genus. Its sori appear on the culms as small fusiform swellings, while *E. eleocharidis* causes no perceptible hypertrophy. Besides, *E. parvum* has spores smaller in average, but more variable in size and shape, than *E. eleocharidis*. The spores of the former also have a thicker and darker epispore.

6. Entyloma microsporium (Ung.) Schroet., in Rabh. Fungi Eur.
   n. 1872.   1874.

On *Ranunculus vernyi* Franch. & Sav., Taipeh, coll. K. Sawada, May 13, 1917; also Apr. 4, 1927.

7. Entyloma senecionis Saw., Jour. Taihoku Soc. Agr. and
   For. **7** : 27.   1934.   (Fig. 2, b)

Sori in the leaves, forming chiefly angular brownish spots, limited by veins, 1–2 mm. long; spores globose to ellipsoid, light yellow, 10–13 μ diam., or 14–17 × 8–13 μ, epispore smooth, 0.6–1 μ thick.

On *Senecio formosana* Kitam, Mt. Nêngkao, Taichung, coll. K. Sawada (*type*), Aug. 4, 1928.

This species is probably identical with *Entyloma bavaricum* Sydow.

8. ENTYLOMA ORYZAE H. & P. Syd., Ann. Myc. **12** : 197. 1914.
   Ectostroma oryzae Saw., Taiwan Agr. Rev. no. 63, p. 107.
   1912.

   On *Oryza sativa* L., Taipeh, coll. K. Sawada, Oct. 20, 1926.

9. BURRILLIA AJREKARI Thirumal., Mycologia **39** : 607. 1947.
   Stereosorus monochoriae Saw., Taiwan Agr. Res. Inst. Rept.
   85 : 45. 1943. (*nomen nudum*)

   On *Monochoria vaginalis* Presl, Taipeh, coll. K. Sawada (type
   of *S. monochoriae*), July 8, 1942.

   The genus *Stereosorus* was founded on the assumption that
   the sorus consists entirely of fertile spores, but the specimen shows
   clearly the presence of sterile parenchymatous cells intermixed
   with the spores.

10. DOASSANSIA OPACA Setch., Proc. Amer. Acad. **26** : 15. 1891.
    On *Sagittaria trifolia* L., Taipeh, coll. H. Sueda, May 28, 1919.

USTILAGINACEAE

11. USTILAGO AVENAE (Pers.) Rostr., Overs. K. Danske Vid.
    Selsk. Forh. **1890** : 13. 1890.

    On *Avena sativa* L., Taipeh, coll. K. Sawada, June 4, 1909.

12. USTILAGO CRAMERI Koern., in Fckl. Jahrb. Nass. Ver. Nat.
    27–28 : 11. 1873.

    On *Setaria italica* Beauv., Taitung, coll. K. Sawada, May 29, 1911.

13. USTILAGO CRUS-GALLI Tracy & Earle, Bull. Torrey Bot. Club
    22 : 175. 1895.

    On *Echinochloa crusgalli* Beauv., Taipeh, coll. K. Sawada, June
    17, 1927.

14. USTILAGO CYNODONTIS P. Henn., Bull. Herb. Boiss. **1** : 114.
    1893.

    On *Cynodon dactylon* Pers., Taipeh, coll. K. Sawada, Jan. 17,
    1944.

    Zundel (6) states that the name of this species should be
    written *U. cynodontis* (Pass.) Curzi. Under the provisions of
    article 58 of the International Rules of Botanical Nomenclature,
    however, the name of P. Hennings is the legitimate one.

15. Ustilago emodensis Berk., in Hooker's Jour. Bot. **3**: 202. 1851.

    Ustilago treubii Solms Laubach, Ann. Jard. Bot. Buitenz. **6**: 79. 1886.

    Elateromyces treubii Bub., České Houby **2**: 33. 1912.

    Ustilago rosulata Syd., Ann. Myc. **10**: 77. 1912.

    Farysia emodensis (Berk.) Syd., Ann. Myc. **17**: 42. 1919.

    Liroa emodensis (Berk.) Cif., Nuo. Giorn. Bot. Ital. N. S. **40**: 263. 1933.

On *Polygonum chinense* L., Taipeh, coll. K. Sawada, Mar. 23, 1919; Tsaosan, Taipeh, coll. Y. Hashioka, Feb. 1932.

Bubák transferred this species to *Elateromyces* because of the presence in the sorus of remnants of host tissue which resemble "elaters" superficially. Sydow accordingly placed it in *Farysia*. Ciferri established the genus *Liroa* based on the same fungus. His genus is separated from *Farysia* by its habit of forming galls on various parts of *Polygonum* and its spore formation in lysigenous cavities. These features, however, do not form a sound basis for generic distinctions and especially fail to separate the genus *Liroa* from *Ustilago*. Ciferri also believed that the sporogenesis in this fungus was typical of the Graphiolaceae. On the contrary, Mundkur and Thirumalachar (4) ascertained that the spore formation conformed to that of the Ustilaginaceae. Their studies on the anatomy of the tumor led them to emphasize the formation of an inverted lunate bed of sporogenous tissue restricted to the apical region of the tumor as the most characteristic feature of the genus. This feature, however, is by no means constant even in the species concerned. The fungus attacks the inflorescence as well as the stem, but such inverted lunate beds of sporogenous tissue can be traced out only in the conical outgrowths on the stem. If the fungus manifests itself only in the floral parts, as is often the case, this diagnostic feature will not be present. Moreover, the occurrence of "elater"-like strands of host tissue is not unusual in other species of the Ustilaginaceae. Certain collections of ovaricolous *Ustilago* on *Polygonum* do have such host remnants intermixed with spores. In *Sorosporium tanganikeanum* Zundel, the "yellowish shreds" mentioned in its original description (7) are also very
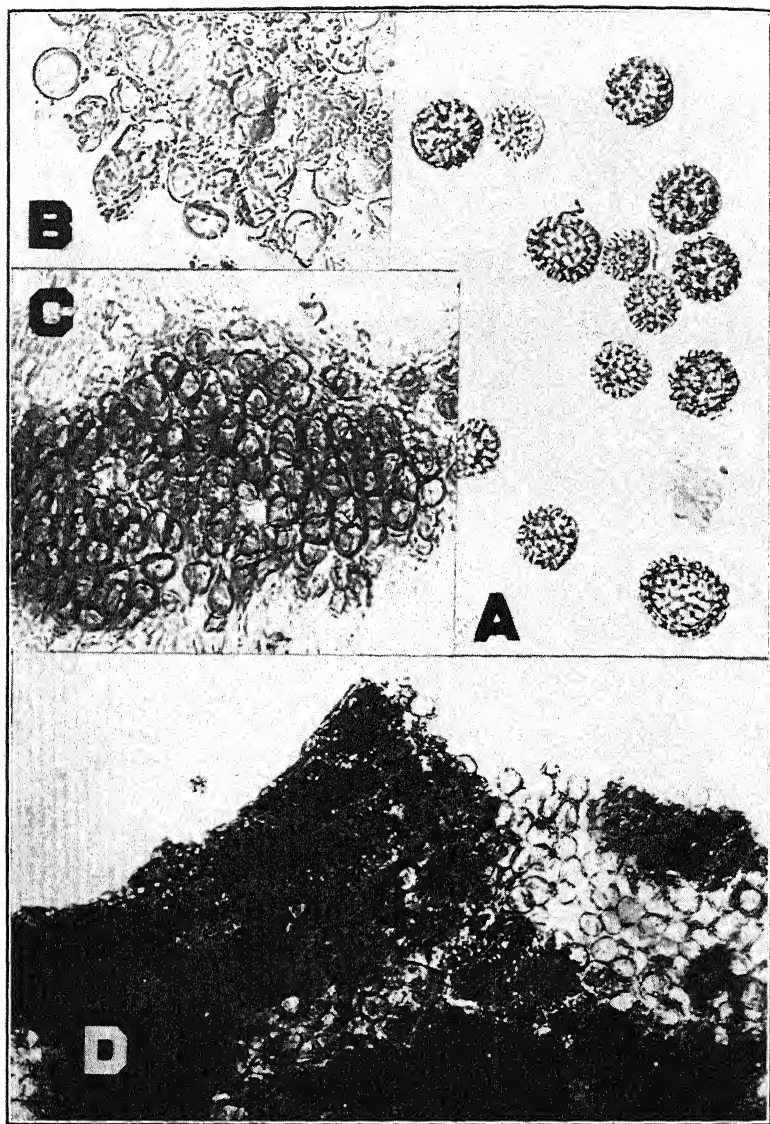
FIG. 2. Ustilaginales of China.

similar to those found in *U. emodensis*. Taking the foregoing into consideration, the proper taxonomic position for the fungus in question is in *Ustilago,* in which genus it was originally placed. Consequently the generic name *Liroa* should be relegated to the synonymy of *Ustilago.*

The spores of this species have been described as smooth, verruculose, or reticulate. All the specimens examined possess spores with very fine and regular reticulations, measuring approximately 0.7–1 μ in width and appearing as verruculae under lower magnifications. In addition to Taiwan collections, the following were included in the present study: PHILIPPINE ISLANDS: Mt. Apo, S. E. Mindanao, coll. R. S. Williams, Apr. 1, 1905; Mt. Puloz, Benguet, Luzon, coll. M. S. Clemens 5127, Feb. 24–27, 1925; Baguio, Benguet, Luzon, coll. M. S. Clemens 51798, Mar. 28, 1935. CEYLON: Hakgala, coll. T. Petch, Aug. 1912 (Syd., Fungi Exot. Exs. 73).

16. USTILAGO ESCULENTA P. Henn., Hedwigia **34**: 10. 1895.

On *Zizania latifolia* Turcz., Taipeh, coll. Y. Hashioka, Nov. 18, 1933; coll. K. Sawada, Nov. 2, 1927.

17. USTILAGO HORDEI (Pers.) Lagerh., Mitt. d. Badischen Bot. Ver. p. 70. 1889.

On *Hordeum sativum* Jessen, Taipeh, coll. K. Sawada, Mar. 8, 1911.

18. USTILAGO KOLLERI Wille, Bot. Notiser **1893**: 10. 1893.

On *Avena sativa* L., Taipeh, coll. K. Sawada, June 4, 1909.

19. USTILAGO KUSANOI Syd., Mem. Herb. Boiss. **4**: 4. 1900.

On *Miscanthus sinensis* Anders., Taipeh, col. K. Sawada, Mar. 25, 1926.

20. USTILAGO NEGLECTA Niessl. in Rabh. Fungi Eur. n. 1200. 1868.

On *Setaria glauca* Beauv., Taichung, coll. Y. Fujikuro, Aug. 8, 1911.

21. USTILAGO NUDA (Jens.) Rostr., Tidsskrift f. Landökonomi **8**: 745. 1889.

On *Hordeum sativa* Jessen, Taipeh, coll. K. Sawada, Mar. 10, 1910.

22. Ustilago scitaminea Syd., Ann. Myc. **22**: 280. 1924.

On *Saccharum officinarum* L., Hsinchu, coll. K. Sawada, June 23, 1929.

23. Ustilago shimadae Saw., Taiwan Agr. Res. Inst. Rept. **87**: 34. 1943.

Soris axem floralem omnino destruentibus et eum in structuram longam, curvatam flagelliformem, membrana albida delicata e contextu hospitis composita tectam, circa 25 cm. longam transformantibus; sporis plerumque globosis usque subglobosis, 4.4–6.5 μ in diam., episporis tenuibus, glabris, pallide olivaceo-brunneis.

In Gramineis indeterminatis.

Sori entirely destroying the floral axis, transforming it into a long, curved flagelliform structure, covered by a whitish delicate membrane of host tissue, approximately 25 cm. long; spores chiefly globose to subglobose, 4.4–6.5 μ diam., epispore thin, smooth, light olivaceous brown.

On an undetermined Grass, Taipeh, coll. Y. Shimada (*type*), Apr. 3, 1911.

The host was noted as *Andropogon?* or *Arundinella?* on the label of the type. The fungus differs from *Ustilago arundinellae-hirtae* Ito, described from Japan, only slightly in spore size.

24. Ustilago shiraiana P. Henn., in Engl. Bot. Jahrb. **28**: 260. 1901.

On *Phyllostachys makinoi* Hayata, Tanshui, Taipeh, coll. K. Sawada, Apr. 16, 1927.

25. Ustilago sparsa Underw., Bull. Torrey Bot. Club **24**: 86. 1897.

On *Dactyloctenium aegypticum* Willd., Anping, Tainan, coll. K. Sawada, Nov. 4, 1908.

26. Ustilago sphaerogena Burr., in Ell. & Ev. N. Am. Fungi n. 1892. 1887.

On *Echinochloa crus-galli* Beauv., Taipeh, coll. K. Sawada, July 5, 1924.

27. Ustilago tritici (Pers.) Rostr., Overs. Kong. Danske Vidensk. Forh. **1890**: 15. 1890.

On *Triticum aestivum* L., Taipeh, coll. Y. Fujikuro, Apr. 2, 1917.

28. USTILAGO TUBERCULIFORMIS Syd., Ann. Myc. **1**: 22.   1903.
  Ustilago foliorum Ito, Trans. Sapporo Nat. Hist. Soc. **14**: 88.
    1935.

On *Polygonum runcinatum* Hamilt. ex D. Don. (= *P. morrisonense* Hayata), Mt. Nêngkao, Taichung, coll. K. Sawada, Aug. 4, 1928.

This species was first collected by A. Henry in Hupeh, Central China.   Efforts to locate the type have been unsuccessful.   The only other record (**5**) is from Darjeeling, India on *Polygonum chinense*.   In this Indian collection made by W. McRae in 1909, the sori occur in the midrib of the leaf, forming conspicuous tumors 3–7 mm. in length.   The spores are light flesh color, 12–14.6 × 11–14.5 μ diam., with violet reticulations of 1.5–2 μ deep by 2–4 μ wide.   The Taiwan collection available for the present study was from the same locality as the type of *U. foliorum*.   The sori are in the leaves causing slight thickening only.   It agrees with the original description of *U. tuberculiformis* even better than the Indian specimen.

29. USTILAGO UTRICULOSA (Nees) Tul., Ann. Sci. Nat. Bot. III.
    7: 102.   1847.

On *Polygonum barbatum* L., Chia-i, coll. K. Sawada, Nov. 9, 1909; on *Polygonum caespitosum* Bl. var. *longisetum* (De Bruyn) Steward, Taipeh, coll. K. Sawada, May 4, 1920; also Dec. 3, 1923; on *Polygonum lapathifolium* L., Taipeh, coll. Y. Hashioka, Apr. 15, 1932; on *Polygonum macranthum* Meisn. (= *P. japonicum* Meisn. var. *conspicuum* Nakai), Shinsha, coll. F. Onuma, Oct. 4, 1930.

Liro (**2**) has seen a collection on *Polygonum barbatum* from Taiwan and referred it to *Ustilago cordai* Liro.

30. SPHACELOTHECA CRUENTA (Kuehn) A. A. Potter, Phytopath.
    2: 98.   1912.

On *Sorghum vulgare* Pers., Taipeh, coll. K. Sawada, June 20, 1911.

31. SPHACELOTHECA HYDROPIPERIS (Schum.) D. By., Verg. Morph. u. Biol. d. Pilze, p. 187. 1884.

  Ustilago polygoni-senticosi P. Henn., *in litt.*

  Sphacelotheca polygoni-senticosi (P. Henn.) Miy. & Tak., Trans. Sapporo Nat. Hist. Soc. **14**: 90. 1935.

On *Polygonum caespitosum* Bl., Taipeh, coll. K. Sawada, May 24, 1917; Taroko, Hualienhsien, coll. T. Hosokawa, Aug. 13, 1932; on *Polygonum caespitosum* Bl. var. *longisetum* (De Bruyn) Steward, Taipeh, coll. Y. Fujikuro, Apr. 3, 1915; Taichung, coll. Y. Hashioka, July 1, 1936; on *Polygonum senticosum* (Meisn.) Franch. & Sav., Wanyülin, Hualienhsien, coll. K. Sawada, May 18, 1919.

32. **Sphacelotheca isachnes** (Syd.) Ling, comb. nov.

  Ustilago isachnes Syd., Ann. Myc. **12**: 77. 1912.

  Ustilago isachnes Saw., Taiwan Agr. Res. Inst. Rept. **87**: 33. 1943.

On *Isachne globosa* (Thunb.) Kuntze, Fêngsan, Kaohsiung, coll. Y. Fujikuro (type of *U. isachnes* Saw.), Oct. 28, 1911.

The spores of Sydow's species were originally described as 8–14 × 7–10 μ in size, while those of Sawada's species were 13–18 × 12–14 μ. A comparison of collections from Taiwan and from the Philippines (Herb. Bureau of Science, P. I. No. 4864), however, revealed a close similarity in their spore size, being 10.5–16.5 × 10.5–13.5 μ and 11.5–17.2 × 10.5–13.5 μ respectively. The spores of the Taiwan collection were also more reddish colored than those of the Philippine collection. Notwithstanding the slight variation, these two collections can be reasonably well referred to one species. The presence of a central columella and a pallid false membrane in the sorus indicates that it belongs to *Sphacelotheca*.

33. SPHACELOTHECA REILIANA (Kuehn) Clint., Jour. Myc. **8**: 141. 1902.

On *Sorghum vulgare* Pers., Taitung, coll. K. Sawada, May 22, 1911.

34. SPHACELOTHECA SORGHI (Lk.) Clint., Jour. Myc. **8**: 140. 1902.

Ustilago sorghicola Speg., Anal. Mus. Nac. Buenos Aires III.
   1 : 58. 1902; Sawada, Agr. Exp. Sta. Gov't Formosa Spec.
   Bull. 19 : 335. 1919.

On *Sorghum vulgare* Pers., Taipeh, coll. K. Sawada, Sept. 20,
1908; coll. Y. Fujikuro, June 30, 1910.

35. SPHACELOTHECA TANGLINENSIS (Tracy & Earle) Zundel,
   Mycologia 36 : 406. 1944.
   Ustilago tonglinensis Tracy & Earle, Bull. Torrey Bot. Club
   22 : 175. 1895.
   Ustilago ischaemi-akoensis Saw., Taiwan Agr. Res. Inst. Rept.
   87 : 33. 1943.

On *Ischaemum ciliare* Retz., var. *genuinum* Hack., Taipeh, coll.
K. Sawada, Jan. 17, 1944; coll. L. Ling, Apr. 9, 1947; on *Ischae-
mum aristatum* L. (probably = *I. akoense* Honda), Fêngsan,
Kaohsiung, coll. Y. Fujikuro (type of *U. ischaemi-akoensis*), Oct.
28, 1911.

The type of this species was collected in Tanglin, Singapore, and
the specific epithet is obviously based on the locality. On the labels
of the specimens of the type collection in both the Mycological Col-
lections, Bureau of Plant Industry, U. S. Department of Agricul-
ture, and the New York Botanical Garden, the specific name and
the locality were spelled "tanglinensis" and "Tanglin" respectively.
In publication, however, both appeared as "tonglin." It is as-
sumed to be a typographic or unintentional error, and, accordingly,
the specific name is corrected here. The following description is
based on the type.

Sori in the ovaries, infecting all the florets in the inflorescence,
3–4 mm. long, concealed by the glumes but later protruding, each
covered by a pallid false membrane which ruptures at maturity,
revealing a dark dusty spore mass surrounding a simple central
columella; sterile cells of false membrane subglobose, oval, or
angular, chiefly in chains, 7.5–20 × 6.7–13 μ; spores reddish brown,
globose, subglobose or ellipsoid, echinulate, 9.5–11.5 μ diam., or
9–12 × 8.7–10 μ.

36. MELANOPSICHIUM PENNSYLVANICUM Hirschh., Notas Mus.
   La Plata, Bot. 6 : 149. 1941.

On *Polygonum lapathifolium* L., Taipeh, coll. K. Sawada, Aug. 4, 1924; on *Polygonum minus* Hudson ?, Taipeh, coll. Y. Hashioka, Aug. 1934.

37. CINTRACTIA AXICOLA (Berk.) Cornu, Ann. Sci. Nat. Bot. VI. **15**: 279. 1883.

   Cintractia fimbristylis-kagiensis Saw., Dept. Agr. Gov't Res. Inst. Formosa Rept. **2**: 79. 1922.

   Cintractia suedae Saw., Dept. Agr. Gov't Res. Inst. Formosa Rept. **2**: 81. 1922.

On *Fimbristylis diphylla* Vahl, Taipeh, coll. H. Sueda (type of *C. suedae*), Nov. 12, 1920; Taichung, coll. Y. Fujikuro, Aug. 6, 1911; Baikei, Taichung, coll. Y. Hashioka, July 3, 1936; Taipeh, coll. Y. Hashioka, Aug. 1934; on *Fimbristylis kagiensis* Hayata, Taichung, coll. Y. Fujikuro (type of *C. fimbristylis-kagiensis*), Oct. 11, 1913.

Cintractia fimbristylis-kagiensis and *C. suedae* were both described as attacking the ovaries. The current taxonomic concept, however, includes such forms under *C. axicola,* though it is very unusual to find in one collection both the florets and peduncles infected. Sawada also noted some difference in spore color between his two species, but it is not shown in the specimens.

38. CINTRACTIA LEUCODERMA (Berk.) P. Henn., Hedwigia **34**: 335. 1895.

   Cintractia albida Ito, Trans. Sapporo Nat. Hist. Soc. **14**: 93. 1935.

On *Rynchospora corymbosa* (L.) Britt., Taipeh, coll. T. Suzuki (type of *C. albida*), May 1932.

Ito states that in *Cintractia albida* the warts on the spore membranes are never arranged in parallel lines as are those of *C. leucoderma,* but, to the writer's knowledge, the latter species has never been characterized in that way.

39. CINTRACTIA MINOR (Clint.) Jacks., Mycologia **12**: 153. 1920.

On *Cyperus distans* L., Taichung, coll. Y. Hashioka, July 2, 1936; on *Cyperus compressus* L., Taitung, coll. K. Sawada, May 21, 1911; Sungsan, Taipeh, coll. Y. Hashioka, Aug. 4, 1933, on *Cyperus malaccensis* Roxb., Taipeh, coll. Y. Fujikuro, Sept. 27, 1917.

40. FARYSIA CARICIS-FILICINAE Ito, Trans. Sapporo Nat. Hist.
    Soc. 14: 91.   1935.

   Farysia pseudocyperi Zundel, Mycologia 23 : 297.   1931.

   On *Carex cruciata* Wahl., Taichung, coll. Y. Kudô and S. Sasaki
(type), Sept. 17, 1919; Mt. Hsinkao, Chia-i, coll. T. Kawakami,
Oct. 14, 1906; Taichung, coll. Y. Hashioka, Nov. 6, 1932.

   Ustilago olivacea (DC.) Tul. f. *pseudocyperi* De T. is a name
given to a fungus on *Carex pseudo-cyperus* L. from Argentina.
Zundel applied this name to a collection on an undetermined mem-
ber of the Cyperaceae made by F. A. McClure in Kwangtung,
China, and transferred it to *Farysia* raising it to specific rank.
Both the collections from Argentina and Kwangtung were exam-
ined and proved to be different.   The Argentina collection (Speg.,
Dec. Myc. Argent. 3) is *Farysia olivacea* (DC.) Syd.

   Ito's description of *Farysia caricis-filicinae* was based on a fungus
identical with the Kwangtung collection, but from Taiwan.   The
hosts of both the Taiwan and Kwangtung collections were deter-
mined by Dr. E. H. Walker as *Carex cruciata* Wahl., which is very
similar to *Carex filicina* Nees.   The name *Farysia pseudocyperi*,
although it has priority, was based on an erroneous identification
and has been applied to two different organisms.   As its applica-
tion has become a source of confusion, it should be rejected as a
nomen ambiguum under the provisions of article 62 of the Inter-
national Rules of Botanical Nomenclature.

   F. caricis-filicinae is similar to *Farysia merrillii* (P. Henn.) Syd.
in the general appearance of the sorus and the spores.   The latter
has slightly larger and darker spores with coarser verrucae.   The
frequency distribution of spore measurements of the three collections
concerned are given below:

F. merrillii (type, E. D. Merrill 4915)

Diameter (μ)	8.04	8.71	9.38	10.05	10.72	11.39	12.06	12.73
Frequency	8	23	26	28	11	2	1	1

Mean = 9.55 μ

F. caricis-filicinae (type)

Diameter (μ)	5.36	6.03	6.70	7.37	8.04	8.71	9.38	10.05
Frequency	1	2	24	29	31	6	4	3

Mean = 7.61 μ

F. caricis-filicinae (F. A. McClure, June 10, 1926)

Diameter (μ)	5.36	6.03	6.70	7.37	8.04	8.71	9.38	10.05
Frequency	2	3	5	18	29	22	14	7

Mean = 8.21 μ

41. Sorosporium andropogonis-aciculati (Petch) Petch, Ann. Roy. Bot. Gard. Peradeniya **5**: 227. 1912.

On *Andropogon aciculatus* Retz., Hsinchu, coll. K. Sawada, June 6, 1917.

42. Sorosporium flagellatum Syd. & Butl., Ann. Myc. **5**: 489. 1907.

On *Ischaemum timorense* Kunth. var. *peguense* Hack., Taipeh, coll. Y. Fujikuro, Apr. 24, 1911.

43. Sorosporium formosanum (Saw.) Saw., Dept. Agr. Gov't Res. Inst. Formosa Rept. **35**: 29. 1928. (Fig. 1, b)

   Ustilago formosana Saw., Trans. Taiwan Nat. Hist. Soc. no. 34, p. 6. 1918.

   Ustilago overeeimi Cif., Nuo. Giorn. Bot. Ital. N. S. **40**: 254. 1933.

   Sorosporium punctatum Malenç. & Yen, Rev. Myc. N. S. **2**: 130. 1937.

   Sorosporium overeeimi (Cif.) Malenç., Rev. Myc. N. S. **10**: 121. 1945.

Sori destroying the inflorescence, 4.5–10 cm. long, first each covered by a pale brown false membrane, which gradually flakes away revealing numerous long, slender threads and the dark spore mass; spore-balls rather permanent, 60–195 × 45–115 μ, reniform or broadly elliptical; spores deep brown, finely punctate, 5–7.5 × 4.5–6.5 μ, inner spores lighter in color.

On *Panicum repens* L., Taipeh, coll. Y. Fujikuro (co-type), Apr. 23, 1907; coll. Y. Hashioka, June 1, 1932; coll. K. Sawada, June 21, 1946.

The following species have been recorded on *Panicum repens* with habitat similar to *Sorosporium formosanum: Ustilago digitariae* (Kze.) Wint., *U. overeeimi, Sorosporium punctatum, S. tanganyikeanum* Zundel, and *S. yoshinagae* Zundel. The last-named species was not available for study. *S. tanganyikeanum* is

readily distinguishable from the others by the presence in the sorus of very fine shreds of host tissue, measuring up to 250 μ in width. The identity between *S. punctatum* and *U. overeeimi* has been discussed by Malençon (3). An examination of the type of *S. punctatum* and a specimen on the same host from Java, where *U. overeeimi* was originally collected, discloses both in agreement with *S. formosanum*.

The relation between *U. digitariae* and *S. formosanum* deserves special consideration. Several collections on *Digitaria* from Europe and Mexico, labelled as *U. digitariae,* were studied and found to possess rather evanescent spore-balls and smooth spores. On the other hand, collections on *Panicum repens* labelled the same from Algeria, Spain, and Ceylon, in spite of some variation in the spore color, may be classified satisfactorily as *S. formosanum*. The only exception is a collection from India on that host, which has spore-balls rather irregular and less permanent than the others. Nevertheless, the name *Sorosporium digitariae* (Kze.) Padw., as based on the Indian collection on *Panicum repens,* should not be used until authentic specimens on the type host, *i.e., Digitaria sanguinalis* (L.) Heist., are studied.

Besides collections from Taiwan, the following specimens on *Panicum repens* have been included in the present study. MAROC: Rapat, coll. G. Malençon (type of *S. punctatum*), May 1934. JAVA: Buitenzorg, coll. R. D. Rands, Aug. 1920. ALGERIA: coll. L. Ducellia & P. Hariot, 1911 (Vestergr., Myc. Rar. Sel. 1522). SPAIN: Montjnich, Barcelona, coll. E. Gros, Aug. 1918. CEYLON: Hakgala, coll. T. Petch, May 1913. INDIA: Zelwal, Mysore, coll. E. J. Butler, Sept. 9, 1904.

44. SOROSPORIUM PASPALI-THUNBERGII (P. Henn.) Ito, Trans. Sapporo Nat. Hist. Soc. 14: 94. 1935.

On *Paspalum scrobiculatum* L., Taipeh, coll. K. Sawada, Mar. 14, 1910; also July 8, 1942.

45. **Dermatosorus** Saw., gen nov.

Soris ovaria destruentibus, pseudomembrana atra crassa coriacea prominenti irregulariter dehiscenti tectis; sporarum glomerulis sporis fertilibus numerosis compositis, et cortice pseudoparenchymatico cinctis; sporis e magnitudine media, germinatione ignota.

Species typica: *Dermatosorus eleocharidis* super *Eleocharis dulcis.*

Sori destroying the ovaries, each covered by a firm, thick, dark-colored pseudomembrane which later ruptures irregularly, revealing the mass of spore-balls; spore-ball consisting of numerous fertile spores and a delicate cortex of pseudoparenchymatous tissue; spores medium-sized, germination unknown.

Type species: *Dermatosorus eleocharidis* on *Eleocharis dulcis.*

Dermatosorus eleocharidis Saw., sp. nov. (Fig. 1, c; and 2, d)

Soris in ovariis, saepius antherarum partes inferiores perdentibus, primo membrana falsa crassa nigra coriacea tectis, qua dein rumpit et massam sporarum glomerulorum detegit; cellulis membranae firme conjunctis, flavo-brunneis, ellipsoideis, ovoideis vel angularibus, 9–16 × 6.5–15 μ, pariete 1.5 μ crasso; sporarum glomerulis sporis numerosis compositis, rectangularibus, ellipticis vel subglobosis, opacis, 112–606 × 88–358 μ diam., cortice delicato sed subpermanenti, pseudoparenchymatico, atro, 15–75 μ crasso cinctis, cellulis corticis sterilibus, angularibus, rufo-brunneis, 3–6.5 μ diam.; sporis laxe connexis, globosis usque ellipsoideis, 6–11 μ diam., praecipue 7–9 μ, episporio rufo-brunneo, 1 μ crasso, levi.

In ovariis *Eleocharidis dulcis* Trin.

Sori in the ovaries, often involving the basal parts of anthers, glumes intact, at first each covered by a firm, thick, black, false membrane, which is composed of yellow brown cells, firmly bound together, ellipsoidal, ovoid or angular in outline, 9–16 × 6.5–15 μ in diameter, with walls 1.5 μ thick, and which later rupture irregularly, revealing an aggregate of spore-balls; spore-balls many spored, rectangular, ellipsoidal or subglobose, opaque, 112–606 × 88–358 μ diam., covered by a delicate but rather permanent cortex of pseudoparenchymatous tissue, 15–75 μ thick, consisting of reddish brown cells, 3–6.5 μ diam., with walls 1 μ thick; spores loosely united, globose to ellipsoidal, 6–11 μ diam., chiefly 7–9 μ, epispore smooth, reddish brown, approximately 1 μ thick.

On *Eleocharis dulcis* Trin., Kangsan, Kaohsiung, coll. Y. Shimada (*type*), April 10, 1919.

As the germination of the spores is not known, the taxonomic position of this genus is uncertain. According to the structure of the sori and the spore-balls, it probably belongs to the Ustilaginaceae and is closely related to *Sorosporium,* from which it differs in the presence of a cortex layer covering the spore-ball.

46. Tolyposporium bullatum (Schroet.) Schroet., Krypt. Fl. Schles. 3 : 276. 1887.

On *Echinochloa crus-galli* Beauv., Taipeh, coll. K. Sawada, Nov. 5, 1927.

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A NEW OLPIDIOPSIS PARASITE OF KAR-LINGIA ROSEA FROM MARYLAND

JOHN S. KARLING [1]

(WITH 17 FIGURES)

This parasite occurred in cultures of *K. rosea* which had been isolated and grown on bits of onion skin in a water and soil sample collected from a stagnant pool in a pig pen near Frederick, Maryland in April, 1948. Within two weeks after the host sporangia had developed in the substratum a large number of them were found to be infected, and in the course of a month the disease had reached epidemic proportions in several of the cultures. In such cultures as high as fifty-two per cent of the sporangia were parasitized, but in others infection was less severe and even lacking. The epidemic began to subside in six weeks, and after two months no trace of the parasite, except its resting spores, could be found.

As far as present studies go, this fungus appears to be an obligate parasite of *K. rosea*. All attempts to infect cultures of *Karlingia* sp., *K. hyalina, K. granulata, Chytriomyces hyalinus, C. aureus, C. stellatus, Asterophlyctis sarcoptoides, Phlyctorhiza variabilis, Nowakowskiella elegans, Septochytrium variable,* and *Cladochytrium replicatum* have failed.

This parasite is characterized by a monocentric, holocarpic thallus; spherical to oval, pyriform, obpyriform, oblong, or slightly angular sporangia; biflagellate, heterocont, reniform zoospores, and asexual, spherical, oval, oblong or angular resting spores. Morphologically and developmentally its thallus, sporangia and zoospores resemble those of *Olpidiopsis* species, but it differs from most members of this genus by its asexual resting spores. How-

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ever, if this genus is interpreted to include species with partheno-genetic or asexual resting spores, as the author (1942) has done, the present parasite belongs in *Olpidiopsis.*

With the exception of the doubtful *Pseudolpidium (Olpidiopsis) Sphaeritae,* this is the only species of the family Olpidiopsidaceae known to parasitize a chytrid, and since it appears to be an obligate parasite of *K. rosea* and differs morphologically from other species of *Olpidiopsis* it is regarded as a new species and named *O. Karlingiae* after its host.

Olpidiopsis Karlingiae sp. nov. Sporangiis solitariis vel numerosis, levibus hyalinis, sphaericis 8–120 μ, ovalibus 15–84 × 20–98 μ, oblongatis, 6–10 × 12–28 μ, pyriformibus, obpyriformibus, aut angularibus, tubulis ex-euntibus 6–12 × 14–18 μ aut 7 × 110 μ. Zoosporis hyalinis minutissime granu-latis, reniformibus 6–6.5 × 10–10.8 μ, cum duobus nonaequalibus flagellis 6.5–7 μ et 11.6–13 μ longo. Sporis perdurantibus hyalinis, levibus, sphaericis 8–22 μ, ovalibus, 9–17 × 15–23 μ, oblongatis, 7–9 × 12–20 μ, aut angularibus; germinatione ignota.

Sporangia hyaline, smooth, spherical, 8–120 μ, oval, 15–84 × 20–98 μ, oblong and slightly curved, 6–10 × 12–28 μ, pyriform, obpyriform or slightly angular when pressed together, with one simple, rarely two and branched, short and broad, 6–12 by 14–18 μ, or long, 7 × 110 μ, discharge tubes which protrude directly through the host cell wall or the exit papillae. Zoospores reniform, 6–6.5 × 10–10.8 μ, and containing 30 to 50 minute granules which are not particularly refractive; heterocont, anterior and posterior flagella 6.5–7 μ and 11.6–13 μ long, respectively. Resting spores spherical, 8–22 μ, oval, 9–17 × 15–23 μ, oblong, 7–9 × 12–20 μ or angular when pressed together, with a hyaline, smooth, 1.8–2.2 μ, thick wall, evenly but coarsely granular protoplasm and one or more vacuoles; germination unknown.

Parasitic in *Karlingia rosea,* Frederick, Maryland.

In general appearance this species is strikingly similar to *Olpidium Rhizophlyctidis* recently described by Sparrow (1948) as a parasite of a variant of the same host, and the two species might be easily mistaken until the zoospores are examined and compared. As shown in figures 1–3, one or several parasites develop in a host sporangium, and in exceptional cases as many as fifty-six have been observed in one host cell. In such instances the size of the para-site is relatively small, depending on the number present. In cases of single infection the subsequent thallus may almost fill the
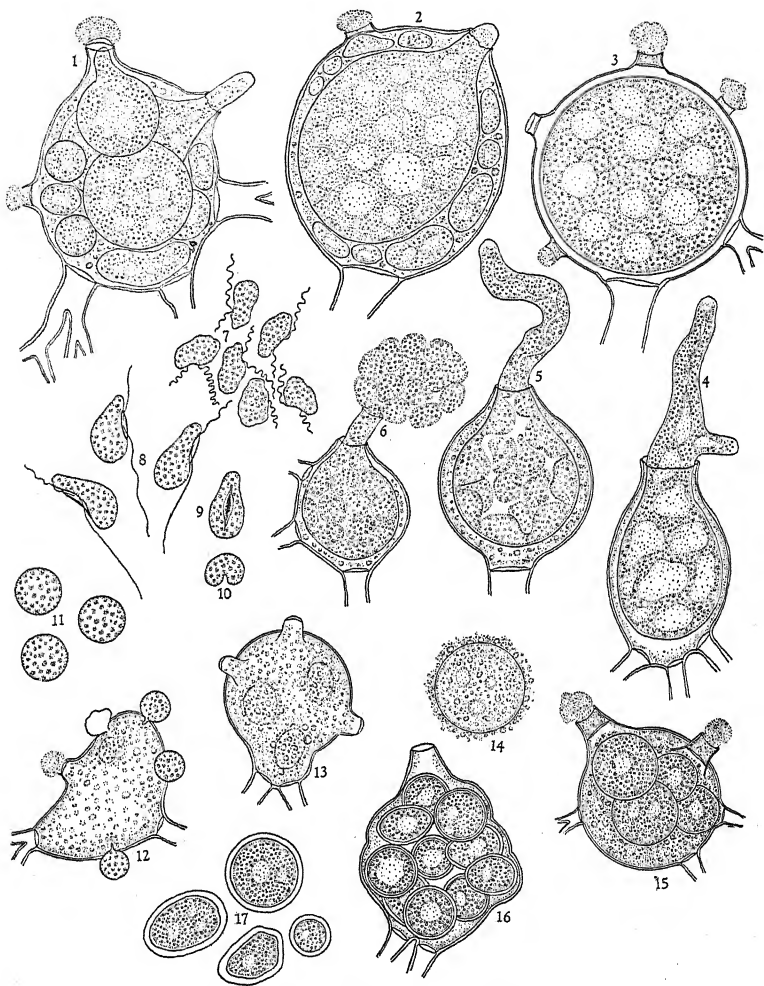
Figs. 1–17. *Olpidiopsis Karlingiae.*

host sporangium (FIG. 3). When multiple infection occurs one or more of the thalli may become relatively large and crowd the smaller ones towards the periphery (FIGS. 1, 2). If several thalli develop equally in size and become large, the resulting sporangia may be distinctly angular in outline from mutual contact and pressure.

The effect of the parasite on the host is fairly constant. No marked enlargement of the sporangia occurs, and only occasionally

are they malformed or distorted. This may occur in cases of multiple infection as the parasites enlarge and cause local bulging of the sporangium wall (FIG. 16). So far no physical antagonism between the host and parasite protoplasm has been observed. The host protoplasm usually aggregates in a dense layer around the parasites (FIGS. 13, 14) without any marked zone of antagonism, as in other species of *Olpidiopsis*. As the parasites enlarge, the host protoplasm is largely absorbed, and finally only a residual layer remains around the mature parasites (FIGS. 1, 2, 5, 6, 15). However, in two cases of single infection only a portion of the host protoplasm was absorbed, while the remainder underwent cleavage and formed normal zoospores.

The method of infection and development of the parasite within the host are fundamentally similar to those of other *Olpidiopsis* species. As shown in figure 12 the zoospore comes to rest on the host cell and forms a short germ or penetration tube through which its content passes into the host protoplasm. The empty zoospore case soon becomes wrinkled, collapses and disappears. The newly-entered parasite is difficult to see in the pink or rosy host proto-plasm, and it is frequently completely obscured. However, as it grows and increases in size it becomes more clearly distinguishable with a well-defined wall, which may often be surrounded by a layer of rosy or reddish, refringent globules from the host protoplasm (FIG. 13). In the young stages the protoplasm of the parasite has a grayish refractive appearance like that of other species of *Olpidi-opsis* and usually includes several refractive bodies. As it increases in size small vacuoles appear in the cytoplasm (FIG. 14), and with continued growth and development these increase in number and size (FIGS. 3, 4). Eventually and usually they coalesce to form one large irregular central vacuole, but in some thalli they may remain relatively small and numerous and retain their entity after cleavage has been completed (FIG. 2) as Barrett (1912) described for *O. vexans*. By this time the protoplasm of the parasite has an even but coarsely, grayish granular appearance (FIGS. 1-4), and the refractive material appears to be highly dispersed. The incipient discharge tube begins as a low broad papilla which then elongates and usually grows out through an exit papilla of the host (FIGS. 4-6). Occasionally, it may perforate and push through the host

wall directly (FIG. 2). As noted elsewhere, it may be short and broad (FIGS. 2, 6) or elongate, curved, rarely branched (FIG. 5), and tapered toward the apex. In mature sporangia with a large central vacuole, the protoplasm usually forms a scalloped layer around the vacuole. Broad V-shaped cleavage furrows form at the border of the vacuole (FIG. 5), progress to the periphery, and thus delimit the zoospore initials. The cleavage segments are well defined at first, but soon become obscured as they increase in size. Shortly after this stage the tip of the discharge tube deliquesces and the zoospore initials flow out (FIG. 6) and form a globular quiescent mass. After a short while they separate and start rocking back and forth as the flagella begin to beat. At this stage the zoospores are somewhat irregular in shape (FIG. 7) without easily recognizable anterior and posterior ends and ventral grooves, but as the rocking continues they mature and assume definitive shape and organization. The rocking movement becomes increasingly rapid, and within 2 to 3 minutes the zoospores swim away. So far no swarming of the zoospores in a vesicle outside of the sporangia has been observed. However, discharge and maturation of the zoospore initials has been seen only a few times, and it is possible that the process and behavior described above may be exceptional instead of general.

The mature zoospore is reniform with a tapering anterior and a rounded posterior end (FIG. 8) and contains from 30 to 50 granules. In the latter respects they resemble very closely those of *Lagenidium Distylae, L. humanum* (Karling, 1944, 1947) and species of *Pythium*. The two unequal flagella are inserted in the ventral groove and extend in opposite directions. In swimming the zoospore rotates slowly on one axis, and the anterior flagellum beats more rapidly than the posterior one, which often appears to be dragged along behind. After swimming about for one-half to three-fourths of an hour, the zoospores slow up, come to rest, retract their flagella and round up (FIG. 11). So far no evidence of diplanetism has been observed, and if the zoospores do not come to rest on a host cell and germinate they degenerate within a few minutes.

As noted previously, the resting spores are apparently formed asexually. No evidence of fusion between so-called male and fe-

male thalli has been found.   Up to a certain stage the development of the incipient resting spore is very similar to that of the sporangia, and the two are difficult to distinguish in the young stages.   However, the wall of the incipient spore soon begins to thicken, and the content becomes more densely granular (FIG. 16).   At the same time the small vacuoles usually coalesce and form a larger central one (FIGS. 15, 16) which may be irregular or spherical in shape and up to $10\,\mu$ in diameter in exceptional cases.   On the other hand, it is not uncommon to find mature spores with 2 or 3 small vacuoles (FIG. 17).   Like the sporangia, the resting spores may occur singly or in large numbers in a host cell (FIG. 16).   In rare cases as many as 43 were found in one host sporangium, which was distorted by the tightly packed spores.   So far germination of the resting spores has not been observed.

In connection with the description of this new species it may be noted that other related members of the Olpidiopsidaceae were found and isolated in soil and water collections from various parts of Maryland.   These are: *Pythiella vernalis* (Couch, **1935**) parasitizing *Pythium* sp. in filaments of *Vaucheria germinata* from a pond near Long Beach, Calvert County; *Olpidiopsis Aphanomycis* parasitic in *A. laevis,* Wicomico River, Charles County and near Centerville, Queen Anne's County; *O. Pythii* in *Pythium* sp., near Centerville, Queen Anne's County; and *O. Achlyae* in *Achlya flagellata,* Old Spout Farm, Calvert County.

In addition to these members of the Olpidiopsidaceae, three species of the family Lagenidiaceae were found and isolated: *Lagenidium pygmaeum* in apple pollen, Solomons Island, Calvert County; *L. Closterii* in *Closterium setiferum* from a pond near Long Beach, Calvert County, and *L. humanum* in dead human skin, Old Spout Farm, Calvert County.

SUMMARY

Olpidiopsis Karlingiae is an endobiotic parasite of the sporangia of the chytrid *Karlingia rosea*.   It developed in a culture of this host which had been isolated on bits of onion skin from a soil and water sample near Frederick, Maryland in April, 1948.   It is characterized by a monocentric holocarpic thallus, spherical, oval, pyriform or oblong sporangia, with a broad, short or long discharge

tube, reniform, biflagellate, heterocont zoospores, and asexual, spherical, oval, oblong or angular resting spores. So far it appears to be an obligate parasite of *K. rosea* since all attempts to infect eleven other chytrids have failed.

In addition to this species seven other related simple holocarpic biflagellate Phycomycetes were isolated from water and soil in various parts of Maryland.

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EXPLANATION OF FIGURES

FIG. 1. Sporangium of *Karlingia rosea* parasitized by 3 large and 6 small thalli of *O. Karlingiae*. FIG. 2. Same host with one large and ten small parasites. FIG. 3. Same host with a single large parasite. FIG. 4. Sporangium of *O. Karlingiae* with numerous irregular vacuoles and a broad, tapering, branched discharge tube. FIG. 5. Centrifugal cleavage in a sporangium with a long curved discharge tube. FIG. 6. Discharge of zoospores. FIG. 7. Maturing zoospores undergoing rocking movements. FIG. 8. Side views of zoospores showing heterocont flagella and ventral groove. FIGS. 9, 10. Ventral and cross section views of zoospores. FIG. 11. Zoospores after coming to rest and rounding up. FIG. 12. Infection stages. FIG. 13. Young parasites within host protoplasm. FIG. 14. Later developmental stage. FIG. 15. Host sporangium with four incipient resting spores. FIG. 16. Distorted host sporangium with ten mature resting spores. FIG. 17. Variations in size and shape of mature resting spores.

CONCERNING THE IDENTITY OF ITER-
SON'S CELLULOLYTIC MYCOGONE

WILLIAM W. DIEHL

In various published discussions of the role of fungi in relation to aerobic deterioration of cellulosic materials there are references to one of the two pioneer research papers by the Dutch microbiologist, Iterson (1, 2), in which among a list of fifteen species of fungi the most active one was reported under the name *Mycogone puccinioides* (Preuss) Sacc. (syn. *Blastotrichum puccinioides* Preuss). Since Iterson's account was published this name has been quoted liberally in various articles concerned with deterioration of fabrics and other cellulosic materials, notably in the well-known compendium by Thaysen and Bunker (3). In that compendium the brief description of *Blastotrichum puccinioides* agrees, unfortunately, with the original description of that species by Preuss (4) rather than with Iterson's records. Preuss's *Blastotrichum puccinioides* was described and illustrated as having septate spores from a mycelium growing on a mushroom and was distinctly different from the amerosporous organism so identified by Iterson (l.c.). A review of the literature of cellulolytic deterioration suggests the likelihood that specific agents variously referred also to species of the form-genera *Acremonium, Acremoniella, Sepedonium, Monotospora*, etc., may well be closely related, if not the same, species as that reported by Iterson, but it is convenient to restrict the present discussion to the identity of Iterson's oft-cited fungus.

Among the cultures frequently obtained from decaying cotton fabrics is a dark-spored hyphomycete which has features suggesting the likelihood of its identity with Iterson's fungus. I first isolated it in pure culture in 1945 from a cotton fabric which had recently suffered decay through use in military operations in the South Pacific area. It was isolated readily using a modification of Iterson's (l.c.) technique in recovering the organism which grew most rapidly following the placing of a bit of the decayed fabric as inoculum upon sterile filter paper lying on a petri-dish plate of sterile

mineral salts agar. With filter paper as the sole source of carbon the black-spored fungus that grew over the filter paper was obviously the active agent in its decomposition. This same fungus had previously been encountered and isolated by P. B. Marsh and Katharina Bollenbacher in the course of studies of the etiology of fabric deterioration. They had isolated it from fabrics after exposure to the elements in various parts of the world and have since recovered it also from cotton fabrics that had been buried experimentally in native soils at Beltsville, Maryland. These isolates, although showing much variation among themselves and incidentally much given to sectoring, appeared enough alike to be considered one species, which is here identified as *Humicola fusco-atra* Traaen (5). This species was originally described from a culture isolated from soil in Norway and has since been recorded from the U. S. by Mason (6) after examination of a culture sent to him by Dr. S. A. Waksman as an isolate from soil in New Jersey. Most curiously this specific name appears to have been accorded little or no attention by workers concerned either with soil microbiology or with problems of cellulolytic deterioration; if the species was encountered, as seems likely, it has been reported under different names. It seems especially odd also that no consideration has been given to Iterson's fungus under the name *Humicola* since Traaen (l.c.) himself suggested that it might be the same as his species *H. grisea*. It appears to me, however, that Traaen's suggestion is confusing because the spore dimensions of his *H. grisea*, "12–17 μ," are very different from those that may be calculated from Iterson's illustrations of his cellulolytic fungus. By measuring the spores as shown by Iterson and calculating their sizes in the light of the stated magnification they measure 7.25–8.18 μ, which is well within the dimensions "6–9–12 μ" as given by Traaen for his *H. fusco-atra*. It is, furthermore, pertinent to note that the cultures of cellulolytic fungi isolated by Dr. Marsh and his associates and by myself, which I have referred to *H. fusco-atra,* are very similar to the culture of *H. fusco-atra* kindly furnished me in 1945 by Dr. S. A. Waksman; this was a transfer from the same original Waksman isolate reported as *H. fusco-atra* by Mason (l.c.) after his comparison of it with Traaen's original type culture.

In addition to its practical bearing on studies of deterioration of cellulosic materials and of soil microbiology this species is of concern nomenclatorially. Mason (l.c.), who has given critical consideration to the two different form-genera *Monotospora* of Saccardo and *Monotospora* of Corda, considered Traaen's *H. grisea* in the former as a synonym of *M. daleae* Mason and *H. fusco-atra* as referable to the latter. He pointed out that, if *Monotospora* of Saccardo should be conserved against Corda's prior name, *Humicola* as a synonym of the discarded *Monotospora* of Corda would then be legitimate with *H. fusco-atra* Traaen as the type species. Because of its convenience this should be a welcome taxonomic disposition of that hitherto debated form-genus.

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COLUS SCHELLENBERGIAE AGAIN

W. C. Coker and G. C. Rebell

(with 1 figure)

Two previous collections of *Colus Schellenbergiae* Sumstine (7), now generally considered as probably identical with *C. javanicus* Penzig (4), have been reported for North America: the type, Pittsburgh, Pa., and one by Dr. Seaver in the New York Botanical Garden (5). The American plant was first illustrated by Dr. Seaver (6, pl. 8) and has not been illustrated since.

The present report concerns two additional collections, as follows.

Miss Jane Miller (now Mrs. Rebell), Summit, New Jersey, July 27, 1938 (Univ. N. C. Herb.). Fourteen sporophores appeared and matured in a few days on a rotted log toward the end of an excessively rainy summer. All sporophores had three arms roundly arched and united at the tips. None were found there the following year or since.

G. C. Rebell, Ridley Park, Pa., Aug. 4, 1948. More than seventy-four sporophores appeared in woods during rainy weather throughout August; growing from rotted wood and humus in an area about 50 by 100 feet, in part marked by the site of a former ornamental garden. A description of these plants follows.

Volva spherical to ovoid, 1–1.7 cm. in diameter, white or dirty brown; stalk an open cup or tube (depending on the length), 0.6–3.7 cm. long, 0.5–1.1 cm. wide at center when flattened, flaring out at the top to support the arms. Arms 3 (in one plant, 2; three plants in Seaver's illustration show four arms), hollow, 1.7–6.8 cm. long, 0.3–0.9 cm. thick at base, tapering upward to attenuated tips that are loosely united and tend to separate after expansion if much disturbed; surface of arms crumpled and pitted, orange-red, paler at base. Gleba dark green, strongly fetid, attached to the inner faces of the arms from the tips to near the base. Spores hyaline, ellipsoid, $1.5–2 \times 4–5(5.5)$ μ.
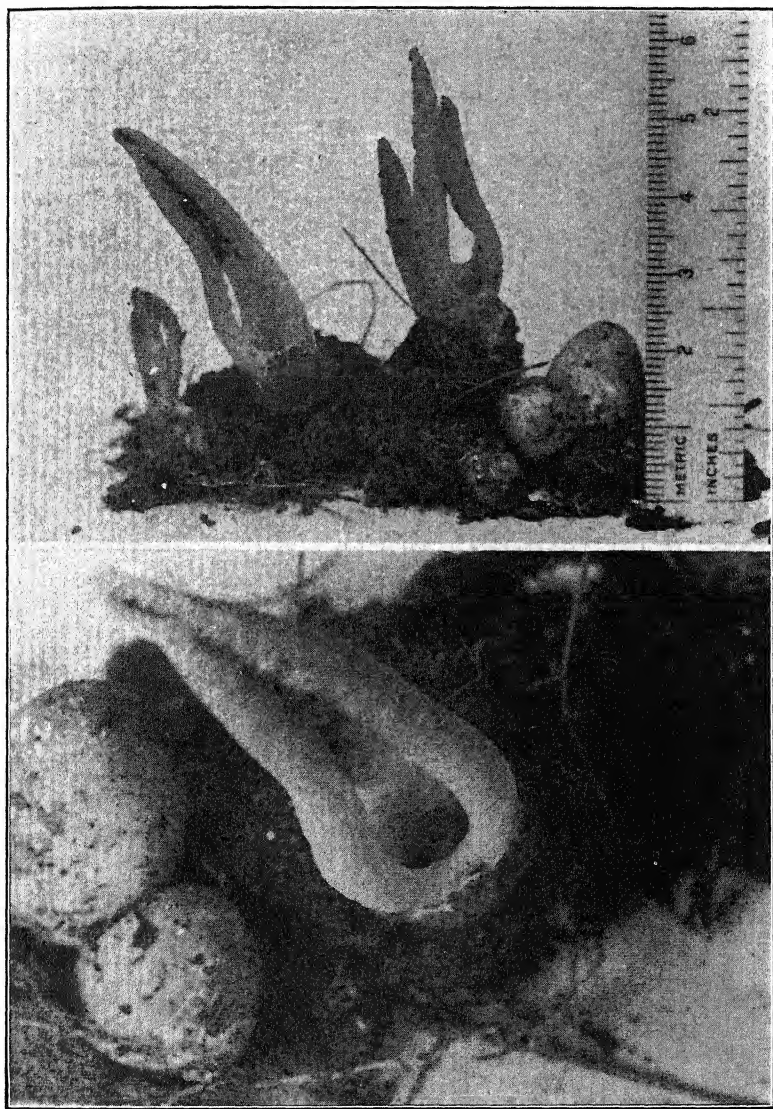
Fig. 1. *Colus Schellenbergiae*. Ridley Park, Pa. Lower figure × 2½.
Photographs by G. C. Rebell.

Like other Phallaceae, the plants tend to vary greatly in size, those developing on dry days being smaller and paler than those developing under wet conditions. Our measurements of 16 plants in fresh condition gave a height, counting stalk and arms, varying from 3.3 to 9.6 cm. as extremes, most of them about 4.6 to 6.4 cm. Sporophores developing in wet weather in the Ridley Park site are larger than those found by Miss Miller in 1938, but mostly smaller than those reported by Sumstine. Most of the stalks are shorter than in the plants illustrated by Seaver. Our spore measurements are narrower than the figures given by Sumstine.

Lloyd's genus *Pseudocolus* (3), it seems to us, may well be recognized for those species which have a stalk with simple arms tapering toward, and united at, the tips. The name would then be *Pseudocolus Schellenbergiae* (Sums.) Johnson (2). The original *Colus* (1) consists of a stalk with arms that form a small latticework at the top. There are several other species with characters similar to the present one, and it would seem convenient to recognize them as a definite group under the genus *Pseudocolus*.

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NOTES ON SOME SPECIES OF PHAKOP-
SORA AND ANGIOPSORA

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(with 3 figures)

In the course of a cursory study of some species of *Phakopsora* deposited in the Pennsylvania State College herbarium, interesting features were noticed in some of the species which warranted a re-study. Comparative studies of the related genera of *Phakopsora* were made in order to have a better knowledge of the morphology of the spore forms for improving our taxonomic concepts of the group.

The genus *Phakopsora* Dietel (1895) was based on *Phakopsora punctiformis* on *Galium aparine* collected in India by Barclay. Dietel (1890), calling the rust a *Melampsora,* emphasized the fact that the teliospore crusts had spores which were not arranged in chains, a condition brought about by younger teliospores develop-ing and pushing into the sorus between older ones (FIG. 3). While this feature of teliospore production is accepted by us, and by most uredinologists, the need for confirmation by examination of the type of the genus still remains. An attempt to locate the type specimen at Kew Herbarium has been unsuccessful. The mode of spore development in chains, or in irregular succession, has been used recently in separating rust genera. Some of the results of studies on the *Phakopsora* species are presented here.

Phakopsora dominicana Kern (1928) was described as a micro-cyclic rust-species on *Croton angustatus.* The material was col-lected in Santo Domingo by Dr. C. E. Chardon. The telia are formed on slightly hypertrophied spots, black, and densely crowded. A careful re-study of the material indicated that the sori are deep-seated developing chains of one-celled, golden-brown teliospores in succession. The telial chains are compactly grouped showing some lateral coalescence, but can be separated by manipulation. The de-veloping chains of spores rupture through the epidermis so that the

sori are erumpent.   The mature spores germinate away at the apex
(FIG. 1).

The characters enumerated above reveal that the rust is a species
of *Baeodromus,* so far known as a microcyclic rust genus on Com-
positae.   The absence of non-erumpent, lenticular telia, with telio-
spores developing in irregular succession, differentiates it from
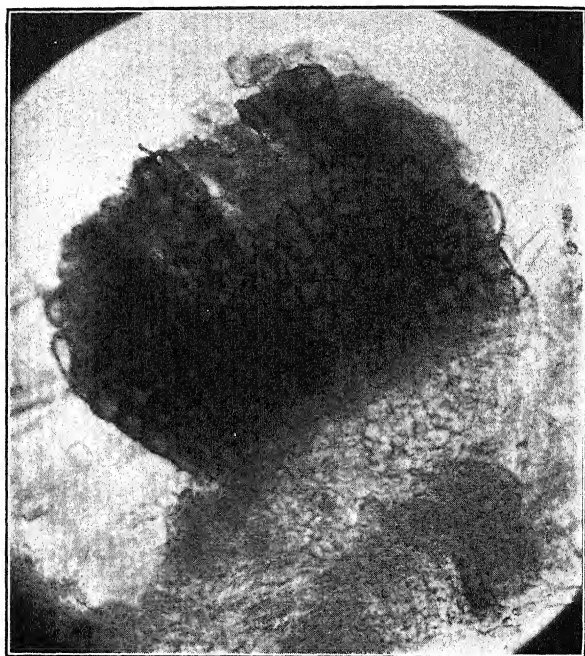
FIG. 1.   Erumpent telium of *Baeodromus dominicana* on *Croton;* note
that the spores are in chains, and that the sorus is deep seated and pro-
truding.

Phakopsora.   The rust is transferred under *Baeodromus* as **B.
dominicana** (Kern) n. comb.

Chains of one-celled teliospores also occur in *Angiopsora, Ar-
thuria, Dietelia, Cerotelium* and others.   Species of *Angiopsora*
and *Arthuria* have been mistaken several times for *Phakopsora* or
Dasturella by workers on rusts.   A brief account of their distin-
guishing characters will be taken up later.

A rust on *Randia armata* from Venezuela was described by

Kern and Thurston (**1944**) as *Phakopsora Randiae*. The uredia are hypophyllous with characteristic incurved paraphyses. The telia are hypophyllous, with 2–6 superposed teliospores forming compact lenticular, non-erumpent crusts (FIG. 2). The spores measure $16–35 \times 10–15 \mu$ with apical thickening of $3–7 \mu$.

A re-study of this rust and comparisons with the descriptions of *Phakopsora melaena* described by Sydow (**1939**) from Ecuador
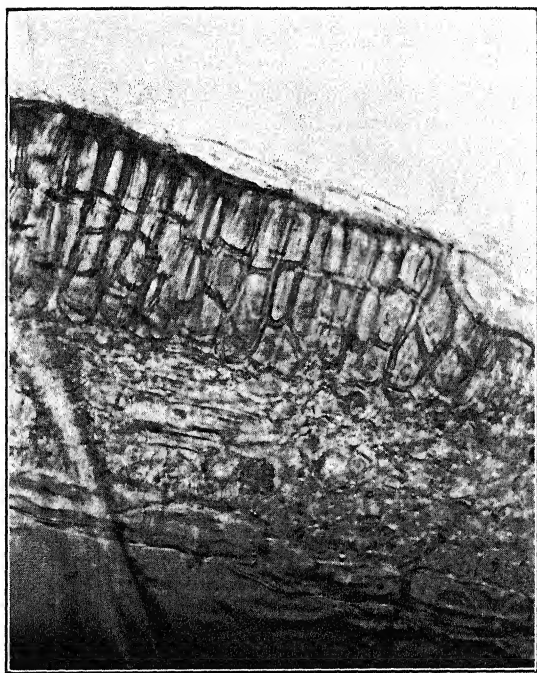
FIG. 2. Indehiscent telium of *Angiopsora melaena* on *Randia* showing teliospores in chains.

revealed that the two rusts are identical and parasitized the same host species. The teliospore measurements given by Sydow are $16–32 \times 14–20 \mu$ with the apical thickening of $3–9 \mu$. The telia are non-erumpent, lenticular; teliospores arranged in chains (FIG. 2). This is a distinguishing character of *Angiopsora* Mains, which differs from *Phakopsora* in the mode of teliospore development. They do not occur in chains in *Phakopsora,* but develop in irregu-

lar succession, the younger spores wedging in between older ones (FIG. 3). This character of chain formation in *Angiopsora* can be clearly seen when spores are of large size, and there are not several spores in a chain. In *A. zeae, A. lenticularis,* and others, the chains are very obvious, but less distinct or obscure in some others, where also they do occur in catenulations. The thinness and plane of sectioning are also of importance in determining the teliospore chains. The rust on *Randia armata* is an *Angiopsora* on account of the occurrence of teliospore chains. Since Sydow's *P. melaena*
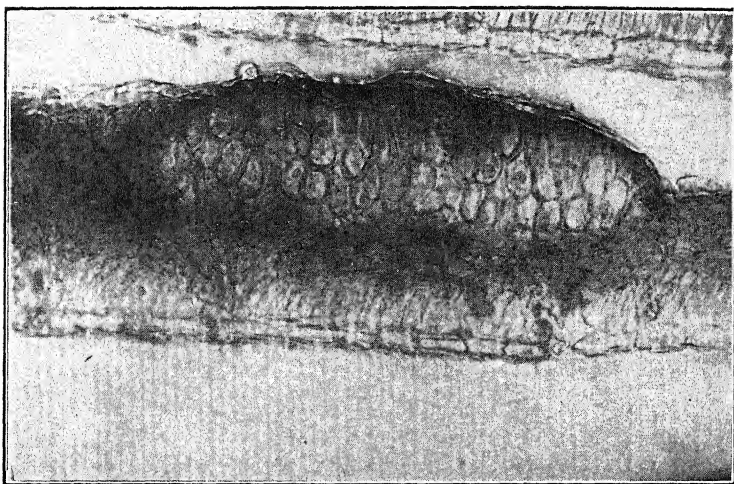
FIG. 3. Lenticular telium of *Phakopsora antiguensis* on *Acalypha* showing the spores in irregular succession, the younger spores developing between the older ones.

was described earlier we propose the following transfer: **Angiopsora melaena** (Sydow) n. comb. (*Phakopsora melaena* Sydow, *P. Randiae* K. and T.)

Examination of the type material of *Phakopsora Hansfordii* Cumm. (1943) on *Alcornea cordifolia* shows that it is also a species of *Angiopsora*. The teliospores are oblong-cuboid, and are in chains of 2–6 spores. The excellent illustration given by Cummins (1943) brings out this character very well. The rust is transferred under *Angiopsora* as **A. Hansfordii** (Cumm.) n. comb.

Phakopsora venezuelana was described by Sydow (1930) on *Sickingia erythroxylonis*. Only uredia were described at first (1930) and later Sydow (1935) found the telial stages in the same collection. The telia are subepidermal, amphigenous, with 2–8 superposed layers of teliospores. Careful examination of telia of *P. venezuelana* revealed that the teliospores are superposed in chains and following our present concept of separating *Angiopsora* from *Phakopsora,* the rust should be placed under *Angiopsora* as **A. venezuelana** (Sydow) n. comb.

Physopella Burserae was described by Sydow (1925) as a leaf rust on *Bursera gummifera* in Costa Rica. The rust was described only in the uredial stage, but taking into account the characteristic incurved paraphyses surrounding the sorus and enclosing sessile urediospores, Sydow placed it under *Physopella*. The genus *Physopella* has been merged with *Cerotelium* or with *Phakopsora* in the absence of telial structures. Several of the species of *Physopella* based upon uredial stages have proved to be *Phakopsora* when the telial stages have been found. *Physopella* (= *Cerotelium*) *desmium* and *P. Cherimoliae,* for instance, have been shown to be species of *Phakopsora* by Cummins.

A careful examination of specimens of *Physopella Burserae* distributed by Sydow (Fungi exotici exsiccati 600, which bears the same date and place of collection as the type specimen) reveals the occurrence of telia. The telia appear microscopically as small brownish-black specks distributed among the uredia. In sections they are lenticular, non-erumpent, with golden-brown teliospores developing in irregular succession and not in chains. Since the telial stage has been discovered, the following change in nomenclature is proposed, **Phakopsora Burserae** (Sydow) n. comb.

Phakopsora Meliosmae was described by Kusano (1904) on the leaves of *Meliosma myrianthae* from Japan. The telia were said to be covered by the epidermis, with 3–4 superposed, polyhedral spores which were thin-walled and light brown. An authentic specimen of *Phakopsora Meliosmae* collected by Kusano, and distributed in "Vestergren's Micromycetes Rarius selecti No. 1558," was carefully sectioned and examined. The uredia are hypophyllous and possess the incurved paraphyses of the *Phakopsora*-type.

The telia are non-erumpent, lenticular, and the teliospores are cuboid and in distinct chains.   The rust is a species of *Angiopsora* and the combination **A. Meliosmae** (Kusano) n. comb. is proposed.

A leaf rust on *Ampelopsis heterophylla,* collected by Shirai near Tokyo, was described by Dietel (**1898**) as *Phakopsora Ampelopsidis* Dietel & Sydow.   In the next year P. Sydow (**1899**) described another species *Phakopsora vitis* on *Parthenocissus Thunbergii.*   The urediospores were stated to be more elongated, and the teliospores larger, in *P. vitis* than in *P. Ampelopsidis.*   Naoharu Hiratsuka (**1900**) made a comparative study of the two and found them to be identical.   He therefore reduced *P. vitis* as a synonym of *P. Ampelopsidis.*   This disposition was maintained in later studies on the *Phakopsora* by Naohide Hiratsuka of Japan (**1935**).   A collection of the rust on *Ampelopsis heterophylla* collected in Japan in 1929, and distributed as *Phakopsora Vitis* (Thüm.) Sydow, has been available for our study.

The uredia are sub-epidermal, erumpent, encircled by incurved paraphyses and open by a pore at the top.   The telia are numerous, appearing as brownish-black specks.   They are non-erumpent, lenticular, with cuboid to globular teliospores occurring in definite chains.   The catenulations of the teliospores are well illustrated by the Sydows (Monographia Uredinearum **3**: 415, Tab. XVII, FIG. 153.   1914).   The rust on *Ampelopsis heterophylla* is an *Angiopsora* according to our present concept of the genus, and is transferred as **Angiopsora Ampelopsidis** (Dietel & Sydow) n. comb.   *Phakopsora Vitis* Sydow is regarded as a synonym.

Studies of *Phakopsora* and related genera indicate that there is some confusion in separating the genera.   Species described as *Phakopsora* have been placed later under *Angiopsora, Arthuria* and *Baeodromus.   Dasturella* was based upon *Angiopsora divina* Syd. Rust species with only uredial stages have been placed under *Cerotelium* or *Phakopsora* on the basis of presence of hyphoid peridium and paraphyses in the sorus.

A concise key which may help in stressing the important characters already enumerated in literature will not be out of place.

KEY

A. Teliospore walls firm, colored, golden-brown to chestnut-brown: telio-
   spores resting.
   (a) Telia indehiscent, lenticular.
       (1) Teliospores in chains...........................*Angiopsora*
       (2) Teliospores developing in irregular succession, younger spores
           alternating with older ones, wedging to form a compact crust.
           (i) Uredinia opening by a pore-like cavity, with encircling pa-
               raphyses developing from hyphoid peridium....*Phakopsora*
           (ii) Uredinia without peridium or paraphyses.........*Bubakia*
   (b) Telia erumpent, protruding, teliospores in chains.
       (1) Telia deep seated...............................*Bacodromus*
       (2) Telia in large flabelliform crusts...................*Dasturella*
B. Teliospore walls thin, hyaline; teliospores germinating at maturity.
   (a) Urediospores in chains.................................*Arthuria*
   (b) Uredia with hyphoid paraphyses; spores borne singly....*Cerotelium*

The genus *Angiopsora* has usually been considered as being re-
stricted to grass hosts. In these studies we have considered eight
species (notes on all species have not been included in this paper)
as follows:

A. Ampelopsidis (Diet. & Sydow) n. comb. on *Ampelopsis hetero-
      phylla.*
A. compressa (Arth. & Holw.) Mains on *Paspalum* sp.
A. Hansfordii (Cumm.) n. comb. on *Alcornea cordifolia.*
A. lenticularis Mains on *Lasiacis* sp. (type).
A. melaena (Sydow) n. comb. on *Randia armata* (FIG. 2).
A. Meliosmae (Kusano) n. comb. on *Meliosma myriantha.*
A. pallescens (Arth.) Mains on *Tripsacum* sp.
A. phakopsoroides (Arth. & Mains) Mains on *Olyria* sp.
A. venezuelana (Sydow) n. comb. on *Sickingia erythroxylonis.*
A. Zeae Mains on *Zea Mays.*

We do not have a complete list of the species of *Phakopsora.*
The following have been examined by us and are placed here with
confidence:

P. antiguensis (Cumm.) K. & T. on *Acalypha* sp. (FIG. 3).
P. Burserae (Sydow) n. comb. on *Bursera gummifera.*
P. Cherimoliae (Lagerh.) Cumm. on *Anona* sp.
P. desmium (B. & Br.) Cumm. on *Gossypium* sp.
P. fenestrala Arth. on *Phyllanthus* sp.
P. jatrophicola (Arth.) Cumm. on *Jatropha* sp. and *Manihot* sp.
P. tecta Jackson & Holway on *Commelina* sp.

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PIGMENT PRODUCTION IN THE DIFFERENTIATION OF TRICHOPHYTON MENTAGROPHYTES AND TRICHOPHYTON RUBRUM

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The two most common fungi concerned in the etiology of dermatophytosis of the feet are *Trichophyton mentagrophytes* (*gypseum*) and *Trichophyton rubrum*. The frequency with which these two species occur was shown by Hopkins *et al.* (1) in a comparative compilation of figures obtained by different workers. Out of 1,700 cultures, 47 per cent were identified as *Trichophyton gypseum* (*mentagrophytes*) and 36 per cent as *Trichophyton rubrum*. Ariyevich and Pentkovskaya (2) reported that the organism most frequently causing epidermophytosis of the feet in U.S.S.R. was, as in most European countries, *Epidermophyton Kaufmann-Wolf* (*Trichophyton gypseum*). *Trichophyton gypseum* (*mentagrophytes*) comprised 94.5 per cent, and *Trichophyton rubrum* 3.2 per cent of 533 cultures they obtained. *Trichophyton rubrum* was the preponderant causative organism isolated by Mu and Kurotchkin (3) from cases of tinea of the feet, hands, nails and body observed in Peiping, China. Out of 134 cultures gathered, 110 were determined as *Trichophyton rubrum*.

It can thus be seen that in the routine work of a dermatomycologic clinic where one of the largest groups of patients are those afflicted with dermatophytosis, the problem of species differentiation of *T. gypseum* and *T. rubrum* is frequently encountered. The desirability of this differentiation has been emphasized in the light of prognostic and therapeutic considerations. In typical cases, *T. gypseum* infections run a more acute inflammatory course and are amenable to treatment while those of *T. rubrum* are of chronic, mildly inflammatory character and resistant to medication. Lewis,

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Montgomery and Hopper (4) considered the chronic nature, dull red color, slight branny scaling and presence of concomitant lesions as suggestive of *T. rubrum* infections while a short, rapid course marked by dyshidrosis and more acute inflammatory reaction is suspicious of *T. gypseum* infection. Diagnosis based on the above clinical criteria, however, cannot be made with certainty as at not a few times, the same kind of lesion may be produced by different fungi and the same fungus may cause several types of lesions. Hopkins *et al.* (1), although agreeing to some extent with the differential points given by Lewis and co-workers, concluded from their experiences that the differences are essentially inconstant.

The final proof for the differentiation of the two types of infection lies in the isolation and identification of the causative organism. At present, the diagnosis is based mainly on the colony characteristics and on morphologic studies in culture mounts. One of us (R. W. B.) (5) listed the distinguishing features as:

Trichophyton mentagrophytes (*gypseum*)	*Trichophyton rubrum*
Honey agar:	
Rapid growth; flat and spreading. Granular to downy surface. White to cream in color. On reverse, rose to brown pigment.	Slower growth; more raised. Granular to fluffy surface. Often shows shades of rose to purple pigment. Wine red on reverse.
Potato dextrose agar:	
Granular surface. Rose brown pigment on reverse.	Sparse aerial growth. Deep wine red pigment on reverse.
Cornmeal dextrose agar:	
No pigment.	Wine red pigment on reverse.
Morphology:	
Spirals; microconidia. Nodular organs. Chlamydospores. Macroconidia on wort agar.	Spirals rare. Microconidia in thyrses. Nodules rare. Macroconidia on wort rare. Macroconidia usually on rice, blood agar, ascitic fluid or dextrose mediums. (In a later study it was shown that heart-infusion agar favored the development of the macroconidia of *Trichophyton rubrum*. Of 50 strains studied on this medium only one failed to produce the characteristic macroconidia.) (R.W.B.) (5a).

These criteria are useful in typical isolates, but again they are notoriously inconstant so that difficulties arise, particularly when, as is not infrequent, pleomorphic and variant forms develop and obscure the cultural picture. The gross appearance of isolates of the same species may vary from one another, and even the same strain may vary with age and with different media. The diagnostic microscopic structures, such as the spirals and the macroconidia, may be absent, and the culture mount then loses its value as an aid for diagnosis. It is because of this inconsistent behavior that no single property can be regarded as reliable for identification.

As its name implies, *T. rubrum* produces a characteristic deep rose purple to wine red pigment best seen on the under side of the colony, and this property has been used as one of its main differentiating points from *T. mentagrophytes*. Lewis and Hopper (6) reported their failure to encounter a single strain that did not acquire a rose purple pigmentation when first isolated. Although the authors were able to maintain the original color of many cultures for three years, they admit that certain strains may lose their pigments after repeated subcultures. This loss of ability to elaborate pigment by certain fungi after the primary isolation is well known and is particularly true for *Trichophyton violaceum*. Another complicating observation that may be a source of confusion was made by one of us (R. W. B.) (5) that some strains with the morphology of *T. mentagrophytes* acquired the rose purple pigmentation characteristic of *T. rubrum*.

It is of interest then to study the behavior of pigment production by *T. mentagrophytes* and *T. rubrum* as an aid in the specific differentiation of these two pathogenic fungi.

MATERIALS AND METHODS

Cultures: The strains utilized were culled from the collections of Dr. Rhoda W. Benham and Dr. Norman F. Conant. Of the 50 strains of *T. mentagrophytes,* 27 were isolated in the Vanderbilt Clinic, 8 were from the stock collection of Dr. Conant and 15 were strains obtained by the Hopkins group during their investigation of epidermophytosis at Fort Benning, Georgia in 1946. Of the 40 *T. rubrum* cultures, 26 were Vanderbilt Clinic strains, 13 were

from Dr. Conant and one was a Fort Benning strain. Most of the strains from the Vanderbilt Clinic were relatively recent isolates.

Media: The composition of the different media used was as follows:

Sabouraud dextrose agar (Difco)

Bacto-peptone .... 10 Gm.
Bacto-dextrose .... 40 Gm.
Bacto-agar .... 15 Gm.
Water, to make ....1000 cc.

Potato dextrose agar (Difco)

Potatoes, infusion from.. 200 Gm.
Bacto-dextrose .... 20 Gm.
Bacto-agar .... 15 Gm.
Water, to make ....1000 cc.

Corn meal agar

Corn meal, infusion from 62.5 Gm.
Agar .... 19 Gm.
Water, to make ......1500 cc.

The chemically pure sugars were added to the amount of 2 per cent.

Blood agar base (Difco)

Beef heart, infusion from 500 Gm.
Bacto-tryptose .... 10 Gm.

Sodium chloride ....   5 Gm.
Bacto-agar .... 15 Gm.
Water, to make ....1000 cc.

Two per cent C.P. dextrose was incorporated.

Casein hydrolysate agar

Casein hydrolysate
  (Smaco) ....   0.05 Gm.
Magnesium sulfate ..   0.1 Gm.
Potassium phosphate.   1.5 Gm.
Dextrose .... 50.0 Gm.
Agar .... 15.0 Gm.
Water, to make 1000   cc.

Ammonium chloride dextrose agar

Ammonium chloride .... 2.5 Gm.
Magnesium sulfate ..... 0.5 Gm.
Potassium phosphate ... 1.5 Gm.
Dextrose ....20.0 Gm.
Agar ....15.0 Gm.

Methods: The different agar media were plated in approximately 10 cc. amounts on small petri dishes of 60 mm. diameter. Inoculations were performed by planting a tiny piece of the fungus colony on the center of the agar plate. The cultures were kept at room temperature and observations made on the first, third and fifth weeks after inoculation. Particular attention was paid to the undersurface pigmentation, the gross appearance and the size of the colonies.

EXPERIMENTAL RESULTS

Pigment Production on Various Media: The 50 strains of *T. mentagrophytes* and 40 of *T. rubrum* were each planted on Sabouraud dextrose agar, potato dextrose agar, corn meal dextrose agar, and ammonium chloride dextrose agar, and the pigment

production noted.   Similar observations were made on heart in-
fusion (blood agar base) dextrose and casein hydrolysate dextrose
agar using 4 selected strains of each species.   Table I summarizes
the results of these observations.

TABLE 1

Undersurface Pigmentation of *T. mentagrophytes* and
T. rubrum on Various Media

Medium	*T. mentagrophytes*	*T. rubrum*
Sabouraud dextrose agar	Yellow to yellow tan to rose tan	Red brown to red purple to wine red
Potato dextrose agar	Cream to light tan to rose purple	Red purple to intense wine red
Casein hydrolysate dextrose agar	Yellow to tan areas	Red purple to wine red
Blood agar base dextrose	Yellowish tan to deep tan with rose tan to rose purple central area	Yellow to yellow tan with purple to purple brown central area
Corn meal dextrose agar	No pigmentation	Red purple to wine red in 95 per cent of the cultures
Ammonium chloride dextrose agar	Cream to yellow	Cream to yellow

On Sabouraud dextrose agar, the early pigmentation of *T.*
mentagrophytes colonies appeared as yellow to yellow-tan to rose-
tan which deepened with age approaching the red-purple color of
T. rubrum in some strains.   The earlier pigmentation of many
strains of *T. rubrum* colonies was not characteristic, ranging from
yellowish cream to dull brown, but it eventually acquired the
characteristic purplish hue.   The color usually started at the
center and gradually spread toward the enlarging edges of the
colony.   The pigment in some cultures became diffuse throughout.
The others left a yellow-tan to rose-tan border.

On potato dextrose agar, the undersurface color of the *T. menta-*
grophytes cultures ranged from cream to light tan to rose-tan, and
even to actual rose-purple in a few strains.   These last five strains
were noteworthy for they acquired, particularly in older cultures on
potato dextrose agar, a pigmentation that approached and was
easily confusable with the red-purple pigmentation of *T. rubrum*.
On this medium, the undersurface pigment of *T. rubrum* was most
intense with a more pronounced purplish tint.

On corn meal dextrose agar, two strains of *T. rubrum* out of 40 produced only a pinkish undersurface coloration, but the rest acquired a red-purple to intense wine red pigmentation which, however, diffused throughout the undersurface only in a few strains. The difference between the two species on this medium is enhanced by the observation that not one of the 50 strains of *T. mentagrophytes* produced any appreciable undersurface pigment.

Pigment Production on Different Sugars: The media used were the basic corn meal agar with the following C.P. sugars incorporated: dextrose, inulin, raffinose, lactose, sucrose, maltose, mannose, galactose and levulose.   Fifteen strains of *T. rubrum* and five of *T. mentagrophytes* were selected and inoculated each on a series of the above corn meal sugar agar media.   Satisfactory growths were obtained in all the mediums except on corn meal inulin agar where the growth was relatively slow and limited.

The red-purple pigmentation produced by *T. rubrum* on corn meal dextrose agar appeared similarly on corn meal agar containing mannose and levulose.   Twelve strains of *T. rubrum* produced no pigment on the media with inulin, raffinose, lactose, sucrose, maltose and galactose.   Three strains acquired a slight pink tinge at the center after five weeks' growth on all the sugar media except corn meal inulin agar.   This might be explained by traces of effective sugars in the corn meal used.

No pigmentation at all was seen on any corn meal sugar medium inoculated with the five *T. mentagrophytes* strains.

Pigment Production on Media Buffered at Different pH Levels: Except for the blood agar base which had a pH of about 6.5, the hydrogen ion concentration of the media ranged from pH 5.5 to 6.0. For this particular experiment, one selected strain of *T. mentagrophytes (gypseum)* and two of *T. rubrum* were inoculated on a series each of Sabouraud dextrose agar, potato dextrose agar, blood agar base dextrose, casein hydrolysate dextrose agar, corn meal dextrose agar and ammonium chloride dextrose agar buffered with phosphate buffers at pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0.

With the exception of corn meal dextrose and the casein hydrolysate agar, essentially the same amount and type of growth and pigmentation as obtained on the unbuffered control was observed on

all the other media in the whole series of hydrogen ion concentrations from pH 4.0 to 8.0. The red-purple pigmentation appeared on Sabouraud dextrose and potato dextrose agar buffered at pH 4.0 as well as at pH 8.0. On corn meal dextrose agar, however, there was noticeable retardation of growth at pH 8.0, especially marked in the *T. rubrum* colonies. The purplish hue of *T. rubrum* failed to develop on corn meal dextrose agar at pH 4.0 and pH 4.5 and on casein hydrolysate dextrose agar at pH 4.0 only cream to yellow pigmentation appeared. The colonies on these two media at the rest of the pH levels acquired the undersurface purple pigment.

REMARKS

The media commonly used and found most satisfactory for the growth of the dermatophytes are highly complex with variable and unknown composition of nitrogenous compounds, carbohydrates, minerals and vitamins, each of which, as well as the proportions in which they occur, may affect in varying degrees the ability of the fungi to produce pigments. The importance of dextrose in the pigment production by *T. rubrum* was demonstrated and emphasized, particularly by Lewis and Hopper (6). Edgecombe (7) was unable to obtain distinct pigment formation with *T. rubrum* on corn meal agar without any added sugar. Likewise, Weidman and Spring (8) failed to develop pigments on Grutz medium with peptone as nitrogen source and glycerin, instead of dextrose, as the source of carbohydrate requirements, but succeeded in doing so abundantly, even with ammonium lactate as the only nitrogen source so long as dextrose was present. They stated that the pigment of *T. rubrum* appeared in the presence simply of nitrogen and dextrose.

On the other hand, the 40 strains of *T. rubrum* here reported produced no pink or red pigment in the presence of dextrose when ammonium chloride was the source of nitrogen. This indicates that not only the type of carbohydrate, but also the nitrogen source determines pigment production. Robbins and Ma (9) in studying the growth factors for *T. mentagrophytes* observed yellow pigmentation in their basic medium with alanine added, reddish brown with

glycine, valine, histidine, and serine and brown with arginine and asparagine.

Several investigators, including Mosher *et al.* (**10**), Robbins and Ma (**9**) and Archibald and Reiss (**11**), have reported significant differences in growth of pathogenic fungi on mediums containing different amino acids or their combinations. Preliminary experiments of our own with a few strains of *T. mentagrophytes* and *T. rubrum* showed that different amino acids and combinations of them used as nitrogen sources do affect pigment production. It is planned to continue the study of this question.

While the presence of vitamins, especially thiamine, is generally accepted as desirable for growth, these essential growth factors do not seem to influence pigment production decisively. Lewis and Hopper (**6**) reported that the incorporation of vitamins A, B complex, B_1, B_2, G, biotin, C and D in a medium with lactose as the only source of carbohydrate did not permit pigment production by *T. rubrum*.

Another factor to be considered in pigment formation is the mineral content of the substrate. Foster (**12**) discussed the relationships between metal assimilation and pigment production by fungi in his review and Nickerson and Williams (**13**) advised investigations of the dermatophytes along these lines.

The results with the different sugars essentially confirm those of Lewis and Hopper (**6**). Three of the monosaccharides tested served to produce pigment, one (galactose) did not; another evidence of the specificity of enzymes for each optical isomer. In their growth experiments with *T. gypseum*, Goddard (**14**) and Mosher *et. al.* (**10**) failed to demonstrate lactase, and Tate (**15**), invertase, zymase or inulase. None of the di- or polysaccharides were utilized for pigment formation although all but inulin aided growth. Inference may be made that the enzyme systems responsible for the elaboration of pigment may be different from those necessary for growth.

Much study has been made of the influence of hydrogen ion concentration on the growth and pigmentation of dermatophytes. Tate (**16**), Goddard (**14**), Peck and Rosenfeld (**17**) and Leise and James (**18**) showed that the dermatophytes they examined were

not fastidious as to their pH environments and can grow quite easily
in a wide range of hydrogen ion concentrations from pH 3.5 to pH
11.0.   Lewis and Hopper (6) were unable to note any appreciable
difference in pigment production by *T. rubrum* when using peptone
crude dextrose agar at pH 4, 5, 6, and 7.   In the present investiga-
tion, changes in hydrogen ion concentration within the range of
pH 4.0 to pH 8.0 also failed to affect notably the pigmentation on
Sabouraud dextrose agar, potato dextrose agar, and blood agar base
dextrose.   This, however, was not true of corn meal dextrose agar
and casein hydrolysate dextrose agar.   As already noted, *T. rubrum*
failed to develop its purple pigment in the most acid of these media,
although it acquired a cream to yellow color.   Nickerson (19) ob-
served the development of a yellow color in a mixed culture of *T.
rubrum* and *Candida albicans* on corn meal agar when the medium
was acid; however, this same strain of *T. rubrum,* when grown
alone on corn meal agar, produced the red color when the medium
was alkaline.   It is noteworthy that the cream to yellow pigmenta-
tion observed at the undersurface of the colonies of *T. rubrum* on a
medium initially at a pH of 4.0 and 4.5 changed to purple or lav-
ender upon dropping sodium hydroxide solution on the colony.
Tate (16) extracted the pigments from *T. rubrum* and *T. gypseum*
and found them to be yellow in acid solution changing to red or
reddish brown in alkaline solution.   Thompson (20) confirmed
Tate's further findings that in alkaline solution the dermatophyte
pigments had a yellow, reduced, and a red, oxidized form.   Both
color changes accompanying differences in pH and in oxidation-
reduction potentials are easily reversible.   Nickerson (19) con-
sidered the possibility that the dermatophytes produce only one
pigment with the pH and the oxidation-reduction potential of the
culture determining the characteristic color.   This is rather intrigu-
ing in the light of the marked variations of the behavior and appear-
ance of pigment production among dermatophytes in the different
media and warrants further investigations.

   As regards the practicality of the results of the present investiga-
tion in the differentiation of *T. mentagrophytes* and *T. rubrum,* the
differences in the undersurface pigmentation of the two species on
corn meal dextrose agar may be used as a diagnostic aid.   It seems

that the purple pigmentation of *T. rubrum* is developed most intensely and characteristically on potato dextrose agar, and to a lesser degree on Sabouraud dextrose agar, as observed by Lewis and Hopper (6) and Edgecombe (7); but on the other hand, atypical strains of *T. mentagrophytes* may acquire reddish pigmentation on these media that may cause confusion. Although the purple pigmentation on corn meal dextrose agar was marked, *T. mentagrophytes* acquired no pigmentation at all while *T. rubrum* developed a red, or at least pinkish pigmentation on the undersurface. This should be a help in differentiating atypical strains of *T. mentagrophytes* and *T. rubrum* when other features are indecisive.

SUMMARY

1. The pigment production of 50 strains of *T. mentagrophytes* and 40 strains of *T. rubrum* was studied on Sabouraud dextrose agar, potato dextrose agar, corn meal dextrose agar and ammonium chloride dextrose agar. It appeared that the appearance of pigment on the undersurface depended not only on the presence of dextrose, but also on the source of nitrogen.

2. The strains of *T. mentagrophytes* examined did not produce any pigment on corn meal dextrose agar while those of *T. rubrum* developed the characteristic red purple color in 38 of the 40 strains examined. The other two strains produced pinkish pigmentation on the undersurface not seen on any strain of *T. mentagrophytes*. This observation is suggested as an aid in differentiation of the two species.

3. *T. rubrum* was able to utilize dextrose, mannose and levulose for pigment production, but failed to do so with inulin, raffinose, lactose, maltose, sucrose and galactose.

4. Pigment production by the strains studied on Sabouraud dextrose agar, potato dextrose agar and blood agar base dextrose buffered at different pH levels from pH 4.0 to 8.0 was not appreciably affected although the *T. rubrum* cultures on casein hydrolysate dextrose agar at pH 4.0 and on corn meal dextrose agar at pH 4.0 and 4.5 acquired only a cream to yellow pigmentation instead of red purple which appeared at other pH levels.

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NEW SPECIES OF TORRUBIELLA, HIRSU-TELLA AND GIBELLULA [1]

E. B. MAINS

(WITH 2 FIGURES)

Torrubiella pulvinata sp. nov. (FIG. 1, A & B)

Mycelium album, corpus hospitis tegente et ad articulos floccos creante; perithecia partim immersa vel superficialia in pulvino mycelii, ovoidea, 450–600 × 220–270 μ, brunnea, a tenui tunica flavi mycelii tecta; asci cylindrici, 450 × 4.5–6 μ, deorsum attenuata, tenuibus membranis, ad apices membranis spissatis 2.5–3 μ; ascosporae filiformes, 0.5–0.7 μ crassae, multiseptatae, cellis 4–8 μ longis; conidiophora e mycelio, sparsa vel caespitosa, 2–2.5 μ crassa, sursum capitulis phialidum; phialides anguste fusoideoovoideae, sursum acuminatae, 8–12 × 2 μ; conidia hyalina, fusoideo-ellipsoidea, 2–3 × 1.5–2 μ, catenulata.

Ex Opilionoideis, Waianuka, Oahu, Hawaii. Dec. 9, 1945, D. P. Rogers (2090).

Mycelium white, covering the body of the host and developing tufts on the legs specially at the joints; perithecia partly embedded or superficial on a pulvinate mass of mycelium, ovoid, 450–600 × 220–270 μ, brown, covered by a thin layer of yellow mycelium; asci cylindric up to 450 μ long, 4.5–6 μ wide, narrowing toward the base, the wall thin, thickened at the apex, 2.5–3 μ; ascospores filiform, 0.5–0.7 μ thick, multiseptate, the cells 4–8 μ long; conidiophores arising from the mycelium, scattered or crowded, 2–2.5 μ wide, phialides grouped in whorls or heads in the upper part of the conidiophores or occasionally from short lateral hyphae, narrowly fusoid-ovoid, acuminate above, 8–12 × 2 μ; conidia hyaline, fusoid-ellipsoid, 2–3 × 1.5–2 μ, catenulate.

On Opilionoidea, south fork of Kaukonahua, 1100 ft., Waianauka, Oahu, Hawaii, Dec. 9, 1945, D. P. Rogers, 2090, type. (Univ. of Mich. and N. Y. Bot. Gard.)

The hosts of this collection are so severely parasitized that accurate determination is difficult. They appear to be arachnids belonging to the Opilionoidea. The conidial stage resembles *Spi-*

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[1] Paper from the Herbarium and the Department of Botany of the University of Michigan.

caria longipes Petch.   In the latter species the phialides generally develop from short hyphal branches.   Petch (**7**) states that *S. longipes* is the conidial stage of *Torrubiella gonylepticida* (Möller) Petch.   *T. gonylepticida* differs from *T. pulvinata* in having the
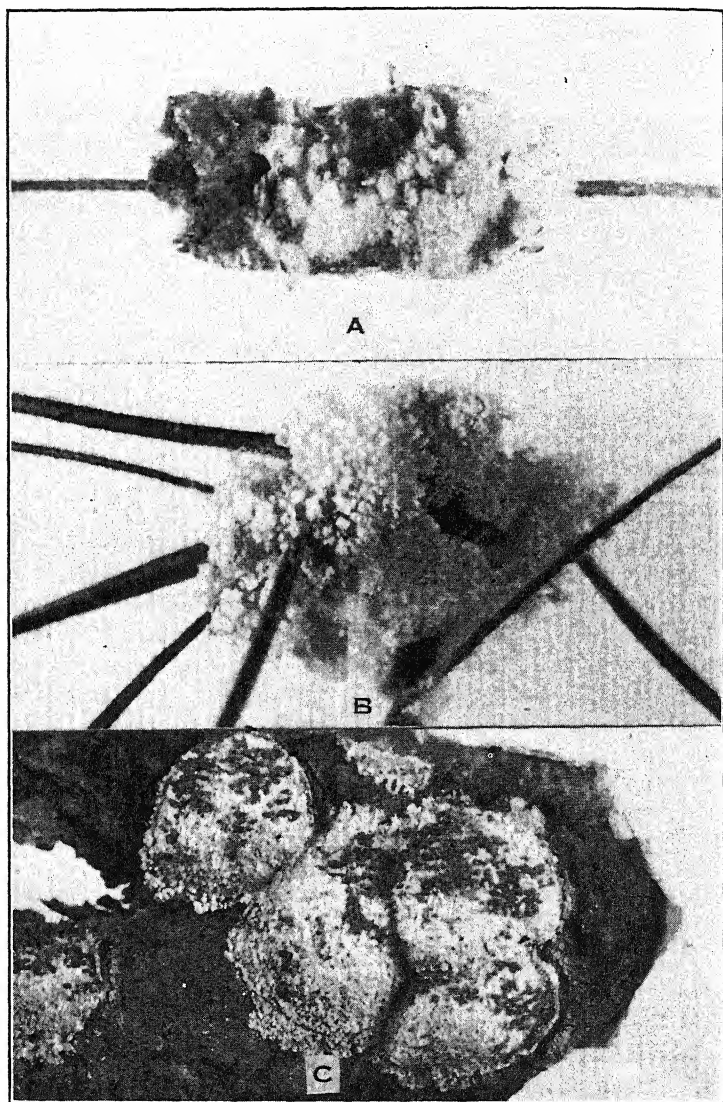
Fig. 1.   *Torrubiella pulvinata* and *T. confragosa.*

perithecia scattered over the entire body and appendages of the host developing from a very scanty superficial mycelium. Möller (2) describes the perithecia of *T. gonylepticida* as orange red, flask-shaped, 300–400 μ long, and the asci as $170 \times 3 \mu$. Since it is desirable to have a name for the conidial stage, **Spicaria pulvinata** is proposed for that of *T. pulvinata*.

Torrubiella confragosa sp. nov. (Fig. 1, C)

Mycelium tenue, album vel cremeum, hospitem tegente, floccosum, pulverulentum; perithecia irregulariter sparsa vel congesta, superficialia vel paululum immersa, ovoidea, 350–650 × 200–375 μ, rubro-brunnea vel castanea, muris 20–35 μ crassis ex duobus stratis constructis, interno strato rubro-brunneo, externo strato brunneo-flavo; asci cylindric, 200–350 × 3.5–4 μ, tenuibus membranis, ad apices membranis spissatis 2–2.5 μ; ascosporae filiformes, multiseptatae; synnemata summe brevia, 300–800 μ longa, 80 μ crassa, cylindricae, interdum furcata; phialides 14–20 μ longae, deorsum 1.5 μ latae, sursum attenuatae, acuminatis apicibus; conidia oblonga, 3–4 × 1.5–1.7 μ, muco tecta, 2–5 in glebulas congregata.

In Coccidis, Novo Petropolis, Brazil, May 1923, Rick, specimen typicum; Bayeaux, Haiti, J. R. Weir.

Mycelium thin, white to cream color, covering scale insects and extending slightly beyond on the substratum, slightly tufted, pulverulent; perithecia irregularly scattered to crowded over the scale, superficial or slightly embedded at the base in the mycelium, ovoid, 350–650 × 200–375 μ, reddish brown to dark chestnut brown, the wall 20–35 μ thick and consisting of two layers, the inner reddish brown, the outer brownish yellow; asci cylindric, 200–350 × 3.5–4 μ, the wall thin, thickened at the apex, 2–2.5 μ; ascospores filiform, 0.5 μ wide, almost as long as asci, multiseptate, part-spores not seen; synnemata very short, 300–800 μ long, 80 μ thick, cylindric or furcate with short irregular branches, consisting of loosely interwoven hyphae; phialides arising from the outer hyphae or from short lateral hyphae, 14–20 μ long, 1.5 μ wide below, gradually narrowing to an acuminate apex; conidia oblong, 3–4 × 1.5–1.7 μ, produced in a mucus, often in clumps of 2 to 5.

On large scale-insects. Novo Petropolis, Rio Grande do Sud, Brazil, May 1923, Rick, **type** (Univ. Mich. and Farlow Herb.); Bayeaux, Haiti, J. R. Weir (Univ. Mich. and Mycol. Coll. Bur. Plant Ind. Washington, D.C.).

Both of these collections were received as *Torrubiella rubra*. *T. rubra* develops perithecia only at the margin of the mycelium cover-

ing the scale or on a hypothallus and the asci are much larger than those of *T. confragosa*. Petch (3) describes a conidial stage for *T. rubra* which differs considerably from that of *T. confragosa*. The latter can best be classified as a *Hirsutella,* although the synnemata are poorly developed. The scattered phialides and clumped spores in a surrounding mucus are characteristic of that genus. Since the conidial stage of fungi of this group frequently develop without the perithecial stage it is convenient to have a name applying to it and consequently **Hirsutella confragosa** is proposed.

Torrubiella liberiana sp. nov. (FIG. 2, A)

Perithecia albido-brunnea, ex myceliis in articulis hospitis erumpentia, partim immersa, ovoidea, 300–400 × 150–200 μ; asci cylindrici, tenues, 210 μ longa, 3–4 μ crassa, tenuibus membranis, ad apices membranis spissatis, 2 μ; ascosporae filiformes, multiseptatae, fragentes, segmentis 3–4 × 0.5–0.7 μ.

Ex formica, Bonata, Liberia, Dec. 8, 1947, J. T. Baldwin, Jr.

Perithecia light brown, developing in small white patches of mycelium at the joints of the appendages and of the body, partly embedded, ovoid, 300–400 × 150–200 μ; asci cylindric, slender, up to 210 μ long, 3–4 μ wide, wall thin, thickened at the apex, 2 μ; ascospores filiform, nearly as long as the asci, multiseptate, breaking into one-celled fragments, 3–4 × 0.5–0.7 μ.

On an ant. Bonata, Central Province, Liberia, Dec. 8, 1947, J. T. Baldwin, Jr. (Univ. Mich.), **type**.

As far as the writer is aware, this is the first report of a species of *Torrubiella* on ants. It occurs in close association with synnemata of a *Hirsutella* which may be the conidial stage. However, it was separated from a collection of ants which were mostly parasitized by *Cordyceps australis*. A *Gibellula* also occurs on a few specimens. The relationship of these fungi is therefore very uncertain and it seems best to describe them separately.

Hirsutella liberiana sp. nov. (FIG. 2, B)

Synnemata pauca, sparsa, ex exiguis albidis myceliis plerumque in articulis erumpentia, albida, tenuia, cylindrica, 3 mm. longa, deorsum 100 μ crassa, sursum paululum attenuata, simplicia vel interdum furcata; phialides sparsae vel congregatae, plerumque nonnullae ex brevibus lateralibus hyphis erumpentes, deorsum fusoideo-ellipsoideae vel fusoideo-oblongae, 8–12 × 3 μ,
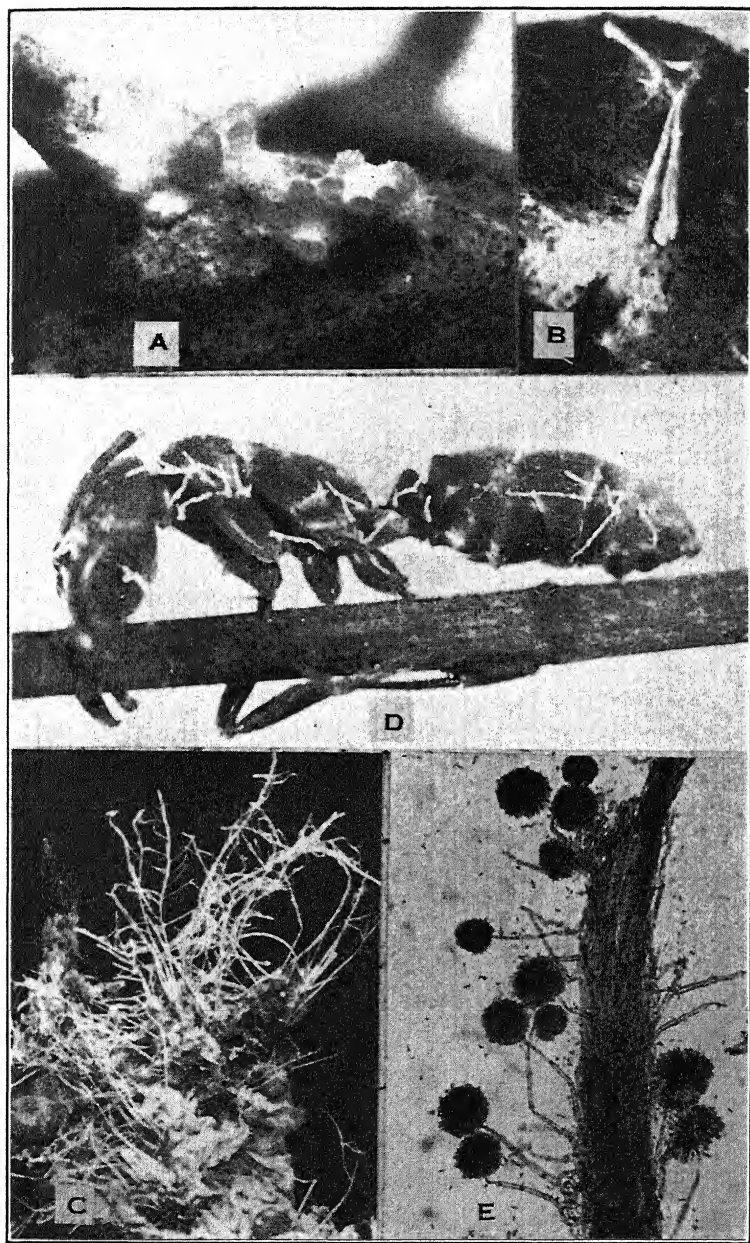
FIG. 2. *Torrubiella, Hirsutella* and *Gibellula*

sursum attenuatae quisque 10–14 μ longo sterigmate; conidia cylindrica, 3–4 × 0.7–1.0 μ; muco tecta, in glebulas congregata.

Ex formica, Bonata, Liberia, Dec. 8, 1947, J. T. Baldwin, Jr.

Synnemata few, scattered, arising from a slight whitish mycelium, usually from the joints of the body and the appendages, whitish, slender, cylindric, slightly narrowing upward, up to 3 mm. long, 100 μ thick, simple or occasionally branched above; phialides scattered to crowded, usually several arising from short, one-celled lateral hyphal branches from the outer hyphae of the synnemata, the lower part fusoid-ellipsoid to fusoid-oblong, 8–12 × 3 μ, the upper part prolonged into an attenuated, acuminate sterigma, 10–14 μ long, each producing several conidia which adhere together in a persistent mucus; conidia cylindric, rounded at both ends, 3–4 × 0.7–1.0 μ.

On an ant, Bonata, Central Province, Liberia, Dec. 8, 1947, J. T. Baldwin, Jr. (Univ. Mich.), **type**.

Petch (6) has described *Hirsutella formicarum* on ants from British Guiana and Ceylon and has stated that it is the conidial stage of *Cordyceps unilateralis*. He describes the conidia as narrowly cymbiform, 9–11 × 2 μ. In an earlier publication (4) he described the conidia of the Ceylon specimens as oval, 3–5 × 1 μ. Kobayasi (1) has identified Japanese collections on ants as *H. formicarum*, describing the conidia as ovoid, ellipsoid or fusiform, 3–4.2 × 1.5–2 μ. It would seem probable that more than one species is concerned. *Hirsutella liberiana* is distinguished by its cylindric narrow conidia. It was associated with perithecia of *Torrubiella liberiana* which is described herein.

Hirsutella ramosa sp. nov. (Fig. 2, C)

Synnemata multa, gregaria, tenuia, irregulariter ramosa, 8 mm. longa, deorsum 52–140 μ crassa, sursum attenuata, accuminatis apicibus, deorsum albido-flava, sursum alba, multis albis rectis vel obliquis ramulis; phialides sparsae vel 1–3 caespitosae, hyalinae, subulatae, 19.3–36.4 μ longae, deorsum 2.1–3.2 μ crassae, sursum attenuatae, acuminatis apicibus; conidia oblonga, 3.2–5.5 × 1.1–1.4 μ, tenui muco tecta, in parvas globosas glebulas 4.3–6.4 μ dia. congregata.

Ex larva lepidopteri, Salmon River, Nova Scotia, Sept. 7, 1931, L. E. Wehmeyer (1474).

Synnemata numerous, arising from all parts of the host, slender, irregularly branched, the main stem up to 8 mm. long, 52–140 μ

thick at the base, gradually narrowing to acuminate apices, light yellow below, white above, the branches numerous, white, at right angles or slightly oblique to the main stem, occasionally producing secondary branches, the hyphae in the stem and branches longitudinal and parallel, multiseptate, 2μ wide; phialides hyaline, minutely roughened, subulate, 19.3–36.4 μ long, 2.1–3.2 μ wide at the base, gradually narrowing to an acuminate apex, scattered on the upper part of the stem and branches giving a setose appearance, arising singly directly from a cell of the outer hypha of the synnema or 1–3 at the apex of short, 6.4–10.7 μ, laterally projecting cylindric hyphae; conidia oblong, 3.2–5.5 × 1.1–1.4 μ, covered with a slight mucus, adhering to form small, 4.3–6.4 μ, spherical clumps.

On fragments of a lepidopterous larva on a mossy log, Salmon River, Nova Scotia, Sept. 7, 1931, L. E. Wehmeyer, **1474, type** (Univ. Mich.).

The much branched synnemata and narrow subulate phialides distinguish *H. ramosa* from other species of *Hirsutella* infecting Lepidoptera.

Gibellula formicarum sp. nov. (Fig. 2, D & E)

Synnemata sparsa, plerumque ex articulis hospitis erumpentia, albido-brunnea, anguste cylindrica, 1–2 mm. longa, 50–150 μ crassa, exterioribus hyphis laxis brunneis asperulatis; conidiophora 50–150 μ longa, 2–4-septata, inferioribus cellulis 3–5 μ latis, asperulatis, superiore cella obovoidea, 7–8 × 4–8 μ, levi, hyalina, globosum vel cylindricum capitulum prophialidium et phialidium gerentis; prophialides ellipsoideae vel subglobosae, 3.5–4 × 2.5–3 μ, quisque paucam phialidem gerentis; phialides ovoideae vel cylindricae, 4–14 × 1.5–2.5 μ; conidia hyalina, cylindrica, 3–4.5 × 1–1.5 μ.

Ex formicis, Belleyella-Kondessu-Zui, Liberia, Dec. 1947, J. T. Baldwin, Jr., specimen typicum; Bonata, Liberia, Dec. 8, 1947, J. T. Baldwin, Jr.

Synnemata scattered, arising from various parts of the body and appendages of the host, usually at the joints, very light brown, narrowly cylindric, 1–2 mm. long, 50–150 μ thick, composed of longitudinal somewhat interwoven hyphae, the outer, loose, brown, asperulate; conidiophores arising from short lateral prolongations of cells of the outer hyphae, 50–150 μ long, 2–4-septate, the lower cells 3–5 μ wide, the walls brownish, asperulate, the terminal cell obovoid, 7–8 × 4–8 μ, smooth, hyaline, bearing a globose to cylindric head of prophialides and phialides; prophialides ellipsoid to subspherical, 3.5–4 × 2.5–3 μ, each bearing several phialides; phialides ovoid to cylindric, 4–14 × 1.5–2.5 μ; conidia hyaline, cylindric, rounded at the ends, 3–4.5 × 1–1.5 μ.

On ants, along the route Belleyella-Kondessu-Zui, Western Province, Liberia, Dec. 8, 1947, J. T. Baldwin, Jr. (Univ. Mich.).

The specimens were included in collections of *Cordyceps australis* (Speg.) Sacc. which were received from Dr. Baldwin. The collections were composites collected from several localities. The synnemata of *Gibellula formicarum* were not found associated with clavae of *C. australis* upon the same insect and therefore the ascogenous stage is uncertain. Petch (5) has reported that the conidial stage of *C. australis* is an *Hymenostilbe*.

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EXPLANATION OF FIGURES

FIG. 1. A & B. *Torrubiella pulvinata.* A. Pulvinate mass of mycelium bearing perithecia arising from a leg of the host. × 12. B. Body of a host covered by a mass of mycelium and conidiophores. × 7. C. *Torrubiella confragosa.* Large scale-insects covered with a thin layer of mycelium-bearing synnemata and perithecia. × 4.

FIG. 2. A. *Torrubiella liberiana* showing perithecia developing on a small mass of mycelia at the base of an antenna of an ant. × 25. B. *Hirsutella liberiana* showing synnemata arising from a small patch of mycelium on an ant. × 7. C. *Hirsutella ramosa* showing the branched synnemata. × 7. D & E. *Gibellula formicarum.* D. An ant showing the scattered synnemata on various parts of the body. × 7. E. A portion of a synnema showing capitate conidiophores (stained with nigrosin). × 200.

PHASE–DETERMINING FACTORS IN BLAS-
TOMYCES DERMATITIDIS

S. B. Salvin

(with 2 figures)

Blastomyces dermatitidis, the causative organism of North Amer-
ican blastomycosis, is characterized by the development (a) of
aerial hyphae with numerous microconidia at room temperature,
and (b) of large thick-walled predominantly single-budding cells
at 37° C. The mycelial phase has been grown on a wide variety of
complex media at room temperature, but the budding or yeastlike
phase has been grown principally on beef-infusion or blood agars at
37° C.

The process of conversion from one growth phase to the other
has been studied since the turn of this century, but is still not
completely understood. Ricketts (8) and Hamburger (4) were
among the first to examine the growth of the fungus "in vitro," and
indicated that temperature was a most influential factor in deter-
mining the morphologic type. This fact has since been confirmed
by others, such as Michelson (7), DeMonbreun (3), and Levine
and Ordal (6). Levine and Ordal (6) also reported pH as being
relatively unimportant in determining the type of growth.

Almost all this work, however, has been done in complex and
not chemically defined media. Hence, little is known about the
possible influence of nutrient factors on the conversion of the or-
ganism from one phase to the other. Investigations were therefore
initiated on the nutritional requirements of the yeastlike and my-
celial phases. This paper reports the effect of growth factors,
amino acids and carbohydrates on the development of *B. derma-
titidis* in the mycelial and yeastlike phases.

MATERIALS AND METHODS

The three strains of *B. dermatitidis* used in the present studies
were from the collection maintained in this laboratory, and were

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originally isolated from cases of human blastomycosis in south-eastern United States. The mycelial phases of the fungi were maintained on Sabouraud's dextrose agar at 25° C., while the budding forms were kept on 20 per cent rabbit blood agar at 37° C.

The basic medium used for the study of the nutritional requirements of the strains under investigation was as follows:

Na_2HPO_4 (anhydrous)	5.0 g.
NaCl	4.0 g.
KCl	1.0 g.
$MgSO_4 \cdot 7H_2O$	0.5 g.
$CaCl_2$	0.025 g.
$FeCl_3$	0.01 g.
agar	1.5 g.
biotin	50 gamma
folic acid	50 gamma
inositol	10 mg.
niacin	1 mg.
calcium pantothenate	1 mg.
p-aminobenzoic acid	10 mg.
pyridoxine	1 mg.
riboflavin	1 mg.
thiamin	1 mg.
distilled water to make	1000 ml.

When the budding phases were to be grown, 1.8 g./liter of a nitrogen-containing compound and 2.5 g./liter of a sugar were added to the above medium. When the mycelia were to be grown, 14 g./liter of a nitrogen-containing compound and 10 g./liter of a sugar were added. All the studies were made in a semifluid medium, since such a medium promoted better development of the yeastlike phase than an agar slant, and also permitted quantitative determination of the growth. In the experiments in which the growth-factor requirements of the fungi were investigated, the agar was washed for a minimum of 5 days in running tap water, then rinsed in distilled water and dried. The medium, after adjustment to pH 7.0, was generally dispensed in 8 ml. quantities in test tubes, and after inoculation with the fungus incubated either at 37° C. in sealed tubes for 2–3 weeks for the budding phases, or at 25° C. for 6–8 weeks for the mycelial phases. The inoculum was either (a) a loopful of a dense washed suspension of microconidia and mycelial fragments in saline, or (b) 0.05 ml. of a washed suspension of about 1,000,000 cells per ml. When necessary, cells were counted in a Levy haemacytometer.

EXPERIMENTS AND RESULTS

When the yeastlike phase of three strains of *B. dermatitidis* was inoculated into 19 tubes of basic medium each one of which also contained 18 amino acids and from each one of which a different natural amino acid was omitted, incubation at 37° C. for two weeks produced good growth of the budding cells. The amino acids studied were glycine, alanine, serine, threonine, valine, leucine, isoleucine, cysteine, methionine, proline, hydroxyproline, tyrosine, tryptophane, phenylalanine, aspartic acid, glutamic acid, lysine, arginine, and histidine.

TABLE 1

GROWTH OF THE YEASTLIKE PHASE OF *Blastomyces dermatitidis* AFTER TWO WEEKS' INCUBATION AT 37° C. IN A SEMIFLUID MEDIUM CONTAINING A SINGLE NITROGEN–BEARING COMPOUND. (Inoculum: 50,000 yeastlike cells/cc.)

Compound*	Number of cells per cc.		
	Strain 6046	. Strain 6021	Strain 6014
ammonium sulfate	75,000	100,000	200,000
glycine	2,100,000	2,250,000	1,275,000
alanine	1,350,000	500,000	500,000
serine	2,350,000	2,125,000	2,150,000
valine	1,000,000	750,000	2,500,000
tyrosine	75,000	100,000	225,000
proline	1,700,000	3,250,000	2,000,000
hydroxyproline	2,750,000	3,500,000	1,875,000
aspartic acid	1,750,000	2,000,000	1,625,000
glutamic acid	1,850,000	2,500,000	3,250,000
arginine	75,000	125,000	200,000

* The media containing threonine, leucine, isoleucine, phenylalanine, cysteine, cystine, methionine, tryptophane, histidine, lysine, or potassium nitrate contained cells in quantities no greater than that in the original inoculum.

In the presence of a single nitrogen-bearing compound (such as one of the amino acids, inorganic ammonium sulfate or potassium nitrate) in the absence of carbohydrate, after inoculation with 50,000 cells of the yeastlike phase and subsequent incubation at 37° C. for two weeks, the amount of growth varied in the different tubes depending upon the nitrogen source (table 1). Extensive development of the yeastlike phase of each of the three strains tested occurred only in the media with glycine, alanine, serine, valine, aspartic acid, glutamic acid, proline and hydroxyproline. Growth

was slight in the tubes in which inorganic nitrogen was the sole source of nitrogen.

In all those cases where the growth was slight, only the yeastlike phase developed. No mycelium or abortive mycelium of any of the three strains studied appeared to any extent during the first
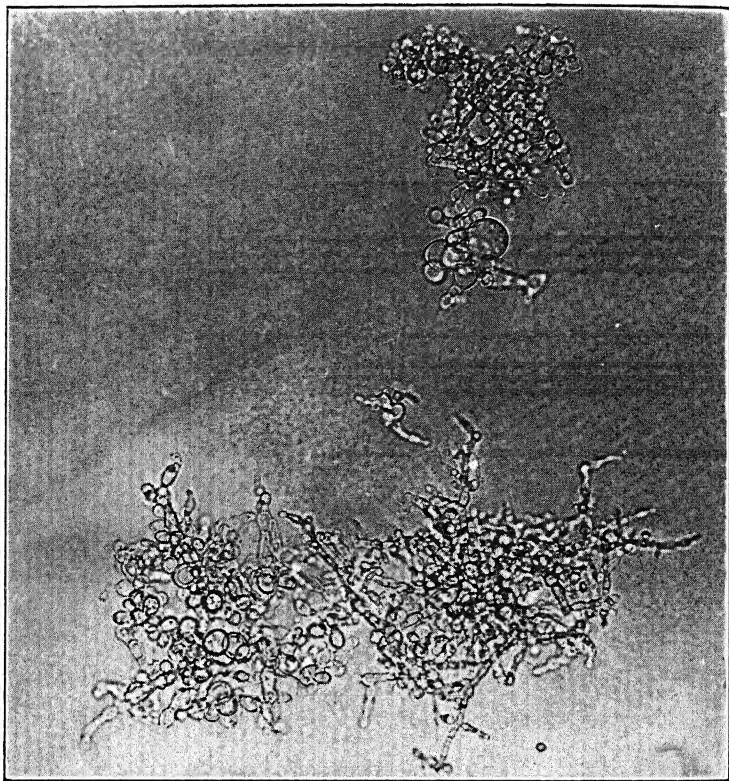
FIG. 1. Abortive mycelium of strain 6014 of *B. dermatitidis* from a culture grown in a peptone semifluid medium for 2 weeks at 37° C.

three weeks of growth at 37° C. in the semifluid medium with pure amino acids. However, abortive mycelia frequently occurred at 37° C. either in a peptone semifluid medium or on 10–20 per cent rabbit blood agar (FIGS. 1, 2).

Tubes containing a single amino acid as the nitrogen source were inoculated with the yeastlike phase of three strains and incubated

TABLE 2

Growth of the Yeastlike Phase of *B. dermatitidis* After 2 Weeks'
Incubation at 37° C. in a Semifluid Medium Containing a Single
Nitrogen–bearing Compound.   (Inoculum: about 50,000
conidia and hyphal fragments per cc.)

Compound*	Number of cells per cc.		
	Strain 6046	Strain 6021	Strain 6014
alanine	25,000	300,000	125,000
serine	2,700,000	3,250,000	2,125,000
proline	50,000	225,000	200,000
hydroxyproline	1,300,000	675,000	925,000

* The media containing the other nitrogen compounds contained less than
25,000 cells per cc.

at 25° C.   Growth was found to occur in varying degrees on all
the amino acids, and was always mycelial in character.

When a suspension of conidia and hyphal fragments was used as
the inoculum in the media containing but a single amino acid and the
culture incubated at 37° C., the yeastlike phase alone developed
where growth did appear (table 2).   However, extensive growth
of the three strains tested occurred only in the media with either
serine or hydroxyproline as the sole amino acids, as well as in the
tubes containing a mixture of twenty different amino acids.   Mod-
erate development appeared in the media with proline or alanine.
In those media where growth was slight, yeastlike cells and no
mycelia were found.

Media were prepared in which glutamic acid was the sole source
of nitrogen and to which one of nine different growth factors
(biotin, folic acid, inositol, nicotinic acid, p-aminobenzoic acid, cal-

TABLE 3

Growth of the Yeastlike Phase of *B. dermatitidis* in a Semifluid Medium
Containing Glutamic Acid as the Nitrogen–bearing Compound and
with Varying Amounts of Dextrose, After 1 Week at 37° C.
(Inoculum: 50,000 yeastlike cells/cc.)

Amount of dextrose (mg./100 ml.)	Number of cells per cc.	
	Strain 6014	Strain 6046
0	250,000	575,000
10	900,000	950,000
100	2,250,000	1,500,000
200	2,850,000	2,300,000
500	5,200,000	2,650,000
1000	4,250,000	2,000,000
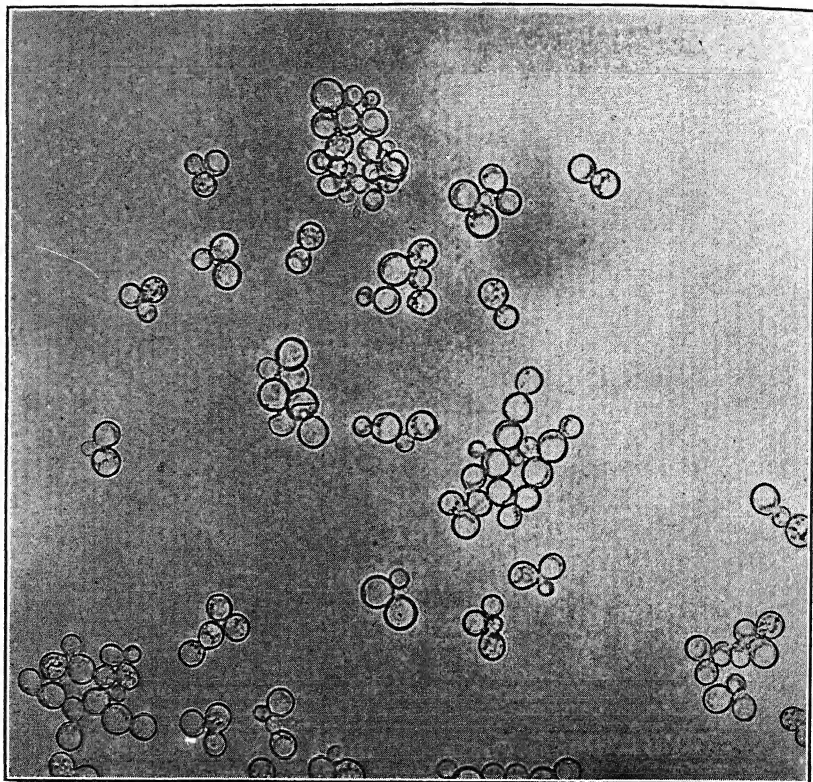
FIG. 2. Budding cells of strain 6014 of *B. dermatitidis* from a 2 weeks' culture (at 37° C.) grown in a semifluid medium with serine as the nitrogen source.

cium pantothenate, pyridoxine, riboflavin and thiamin) was added. Examination after two weeks' incubation at 37° C. revealed about the same number of yeastlike cells in the different media, with no growth factor having any discernible stimulatory effect on the three strains tested.

The yeastlike phase was inoculated into media with glutamic acid as the nitrogen source and with the carbohydrate dextrose varying from 0 to 1000 mg per liter. Budding cells of the yeastlike phase again were the only ones to appear at 37° C., with the optimum concentration of dextrose being 500 mg. (table 3).

When this amount of carbohydrate (5 g./l.) was used in similar

media, and one of 28 different sugars substituted for the dextrose, there was a marked difference in the extent of growth, although some did appear in all the sugars. However, what growth did appear was yeastlike.

DISCUSSION

The phenomenon of dimorphism, whereby a single species can appear in two distinct morphologic phases, is quite common among the pathogenic fungi. For example, *Histoplasma capsulatum* appears in the yeastlike phase at 37° C., and in the mycelial at 25° C. *Blastomyces brasiliensis* develops as a multiple budding cell at 37° C., and as a mycelium at 25° C. *Coccidioides immitis* occurs as an endospore-forming spherule in the tissue, and as mycelium at 25° C.[1] *Candida albicans* has been grown in both the yeastlike and mycelial phases at both 25° C. and 37° C., with the most influential morphology-determining factor or factors still in doubt. *Phialophora verrucosa* appears as round, dark brown bodies in the tissue, and as mycelium in culture.

In some species, such as in *Phialophora verrucosa* and *Coccidioides immitis,* the mechanism is not understood. In others, such as *Candida albicans* and *Histoplasma capsulatum,* some experimentation has provided a partial explanation of the processes involved. The yeastlike phase of *H. capsulatum* does not always occur at 37° C., but must have certain other factors present (9). (a) Biotin must be an ingredient of the medium. (b) A reduced-sulfur group such as the sulfhydryl group must be included. Thus, conversion of the yeastlike phase to the mycelium, and vice versa, may be brought about at 37° C. by changing the amino-acid constitution of the medium.

Evidence has been accumulating that temperature is the principal controlling factor in *B. dermatitidis* (2, 3, 6). However, all this work, with the exception of some brief studies by Levine and Ordal (6) in which ammonium sulfate was the nitrogen source, has been done in complex media the exact chemical composition of which was not known. Such media included primarily various beef

[1] The tissue or spherule phase has not been grown extensively "in vitro" (1, 5).

infusion, peptone or blood agars, and did not permit proper evaluation of the possible effect of the nutrients present in the medium.

In simple chemically-defined media, however, temperature proved still to be the main factor determining growth of mycelia and conidia, or growth of large budding cells. At 25° C., growth was always mycelial; at 37° C., growth was yeastlike, or occasionally consisted of abortive mycelium (FIG. 1). No growth factor was found capable of either (a) stimulating growth of either phase or (b) stimulating conversion of one phase to the other. Although the yeastlike phase developed slightly in the medium containing the ammonium ion as the sole nitrogen source, optimum growth appeared in a medium with amino acids. Glycine, alanine, serine, valine, glutamic acid, aspartic acid, proline and hydroxyproline stimulated development most.

These phase-determining characteristics of *B. dermatitidis* are much like those of *B. brasiliensis* (10). (a) The yeastlike phase of *B. brasiliensis* appeared at 37° C. in the amino-acid media, with glycine, serine, glutamic acid, aspartic acid, proline and hydroxyproline producing most growth. However, growth was much slower, more limited in extent, and reverted more readily to an abortive-mycelium type. (b) Serine and hydroxyproline proved the most stimulatory of the amino acids, especially in converting conidia to budding cells of the yeastlike phase at 37° C. (c) No growth factor was found to have any effect at 37° C. (d) Growth occurred with or without carbohydrate in the medium. This similarity in growth characteristics between *B. dermatitidis* and *B. brasiliensis* parallels their morphologic similarities, and adds further evidence to the beliefs of Conant and Howell (2) that the two organisms belong in the same genus and are very close phylogenetically.

It now seems certain that the morphology of *B. dermatitidis,* as well as of *B. brasiliensis,* is controlled primarily by temperature changes. *H. capsulatum,* however, is determined not only by temperature but also by nutrient factors in the medium. Studies are being conducted to determine what factors are most effective in regulating the phases of other dimorphic pathogenic fungi.

SUMMARY

The yeastlike phase of *B. dermatitidis* was grown in a chemically-defined semifluid medium. Temperature was the most important environmental factor controlling the type of growth. No amino acid, carbohydrate or "growth substance" tested was found to be an essential accessory factor for growth of the yeastlike phase at 37° C. However, the extent of growth of the yeastlike phase did vary greatly according to the constitution of the medium.

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THE PERFECT STAGE OF THE FUNGUS CAUSING SPOT ANTHRACNOSE OF ARBUTUS UNEDO L.

G. Arnaud and A. A. Bitancourt

(with 1 figure)

As shown by Jenkins [1] the causal fungus of spot anthracnose of the strawberry-tree *Arbutus Unedo* L. was first described by P. A. Saccardo and D. Saccardo [2] under the name of *Illosporium Mattirolianum* and included in D. Saccardo's "Mycotheca italica" under number 798. Briosi and Cavara included in their exsiccati "I funghi parassiti delle piante coltivate od utili" under number 349 a specimen of the same organism and identified it as *Hadrotrichum Populi* Sacc. forma *Arbuti*. Jenkins (l.c.) studied and illustrated both specimens and showed that the fungus belonged in the genus *Sphaceloma* and accordingly made the new combination *S. Mattirolianum* (Sacc. & D. Sacc.).

Some time ago, in a routine examination of Briosi and Cavara's specimens in their set of fungi exsiccati (l.c.) in the "Centre de Recherches Agronomiques," Versailles, France, the senior author found on the lesions of number 349 several ascomata of *Elsinoë* in the vicinity of the acervuli and sporodochia of the *Sphaceloma*.

The writers have now completed a more detailed study of this ascomycete which has enabled them to prepare the following description:

Ascomata are seen on the surface of the leaf spot as small, buff colored prominences; in transverse section they are intraepidermal, erumpent, covered with the remnants of the outer wall of the leaf epidermis, and made up of a dark pseudoparenchyma, darker in the upper layers, which form a more or less well-defined epithecium, lighter colored in the deeper layers, which are almost hyaline at the

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[1] Jenkins, A. E. 1933. Additional studies of species of *Elsinoë* and *Sphaceloma.* Mycologia 25 : 213–220.
[2] Saccardo, P. A. 1902. Sylloge Fungorum 16 : 1093.

base and are seated directly on the upper part of the cells forming the palisade parenchyma of the host.   They are 80–50 μ in diameter and 40–60 μ thick.   The asci are distributed in one layer in the upper part of the ascoma and are ovoid or club-shaped, averaging 30 × 18 μ.   The ascospores are hyaline, 1-septate—probably on account of being immature—with the upper cell shorter and broader than the lower cell, constricted at the septum, and usually measure 11 × 4 μ.
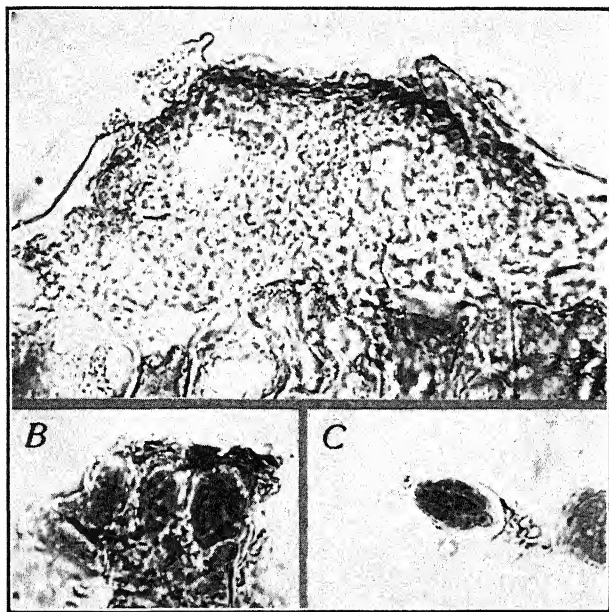
FIG. 1.   *Elsinoë Mattirolianum.*   A, ascoma; B, three asci in place in an ascoma; C, ascus with ascospores.   × 500.   Photo. A. A. Bitancourt.

In all the fungi of which the imperfect stage belongs in the genus *Sphaceloma* and the perfect stage is known, the latter pertains to the genus *Elsinoë.*   The presence on the same lesions of acervuli and sporodochia of *Sphaceloma* and of ascomata of *Elsinoë* is at least circumstantial evidence that both are part of the life cycle of the same organism.   It appears therefore legitimate to consider the ascomata found in the lesions of the spot anthracnose of *Arbutus Unedo* as those of *Sphaceloma Mattirolianum.*

Elsinoë Mattirolianum sp. nov.

Ascomatibus intraepidermicalibus erumpentibus, 80–150 × 40–60 μ pseudo-parenchymatosis e cellulis superne nigricantibus, epithecium formantibus, interne pallidioribus constitutis. Ascis ellipticis octosporis, 30 × 18 μ; asco-sporis hyalinis, uniseptatis (immaturis?) medie constrictis, 11 × 4 μ.[3]

Status conidiiferus:

> Sphaceloma mattirolianum (Sacc. & D. Sacc.) Jenkins (Mycologia **25**: 214. 1933).

> Syn. Illosporium mattirolianum Sacc. & D. Sacc. (Sacc. Syll. Fung. **16**: 1093. 1902).

> Hadrotrichum populi Sacc. forma Arbuti Briosi & Cav. (Fung. parass. piante colt. od ut., number 349), cum ic. 1900.

Material of the perfect stage examined: On leaves of *Arbutus Unedo* L. associated with the imperfect stage as collected at Bari, Italy, by Montagna and distributed by Briosi and Cavara (Fung. parass. piante colt. od ut., number 349) under the name *Hadrotrichum Populi* forma *Arbuti*.

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[3] Translated in Latin by D. B. Pickel.

NOMENCLATORIAL PROBLEMS IN THE PERONOSPORACEAE [1]

CHARLES GARDNER SHAW [2]

(WITH 2 FIGURES)

Whether Article 57 of the International Rules of Botanical Nomenclature (2, 3), as now worded, applies to the Phycomycetes or not has been a subject of considerable controversy (1, 6). The first paragraph of Article 57, which consists of two sentences, is the primary cause of this controversy.

The English version of the first sentence reads as follows: "Among Fungi with a pleomorphic life-cycle the different successive states of the same species (*anamorphoses, status*) can bear only one generic and specific name (binary), that is the earliest which has been given, starting from Fries, *Systema,* or Persoon, *Synopsis,* to the state containing the form which it has been agreed to call the perfect form, provided that the name is otherwise in conformity with the Rules." I consider this sentence to contain the intent of the article and to be unequivocal.

The second sentence of the first paragraph merely defines the perfect state of certain groups of the fungi. It reads as follows: "The perfect state is that which ends in the ascus stage in the *Ascomycetes,* in the basidium in the *Basidiomycetes,* in the teleutospore or its equivalent in the *Uredinales,* and in the spore in the *Ustilaginales.*" That the Phycomycetes are not mentioned in this second sentence, in the absence of evidence to the contrary, must be considered an unintentional omission, which in no way influences the meaning of the first sentence. No other interpretation of the article, as now worded, seems possible to me.

In the Phycomycetes, and particularly in the Peronosporaceae, the "perfect state" has been used sparingly in classification. With few exceptions identifications can be made, and normally are made, on the imperfect or asexual stages alone. Indeed, in the absence of conidiophores and conidia, species of the Peronosporaceae have been placed in the genus *Protomyces* [*e.g. Protomyces graminicola* Sacc. = *Sclerospora graminicola* (Sacc.) Schroeter; *Protomyces fuscus* Peck = *Plasmopara pygmaea* (Unger) Schroeter].

Numerous mycologists, perhaps the majority, would prefer not to apply Article 57 to the Phycomycetes. Bisby (1) has proposed a rewording of this article which would exclude the Phycomycetes. Bisby (2, p. 89) enounces that a proposal, once made, can be followed "unless and until Congress decides against a proposal now before it." I would interpret the Rules differently.

It seems to me that Bisby's proposal and all others are simply recommendations which have no standing according to the Rules until acted upon by an International Botanical Congress. If accepted, they still remain on trial until sanctioned, emended, or rejected by the second successive Congress (Art. 74). Since there is no assurance that any given proposal will be accepted or even acted upon by the next Congress, and since there is no absolute certainty as to when the Congress will again convene, the interests of nomenclatorial stability (Art. 4) are not furthered by following proposals before they are considered by the Congress.

Before any change in the Rules is adopted, consideration should be given to the effect the proposed change would have on the nomenclature of the groups concerned (8). The purpose of this paper is to point out (1) the proper binomials and author citations for certain species of the Peronosporaceae according to the present wording of Article 57 (these binomials are used as headings for the species discussed); (2) the binomials and author citations in common use for these fungi; (3) the binomials that would be valid if Bisby's proposed change in Article 57 were incorporated into the Rules.

The lists of synonyms that follow are not intended to be complete. Only those pertinent in answering the problems indicated above are included. Each synonym is listed exactly as given in the original paper cited. Familiarity with the Botanical Rules of

Nomenclature should make apparent the proper binomials and author citations, either according to the present wording of Article 57 or according to Bisby's proposal. Discussion has therefore been limited to those cases of special interest.

Finally it must be pointed out that, in a discussion of nomenclatorial problems, the distinction between taxonomy and nomenclature is important (8). All decisions about status, whether they concern individuals or major groups, fall within taxonomy. Nomenclature relates only to the choice of names to be used after these taxonomic decisions have been made. The two are diametrically opposed—nomenclature aims at stability; taxonomy, from its very nature, is everchanging.

The present paper deals with nomenclatorial problems. A discussion of taxonomic problems, especially the species concept within the Peronosporaceae, is reserved for a later paper.

1. *Basidiophora Kellermanii* (Swingle ex Saccardo) Wilson— Usually cited as *B. Kellermanii* (Ellis & Halsted) Wilson.

> *Peronospora Kellermanni* Ellis & Halsted (pro tem.) in Ellis & Everh., N. Amer. Fungi No. 2201. 1889. (Nomen nudum.)
> *Plasmopara* sp. Swingle in Trans. Kans. Acad. Sci. **11**: 74. 1889. (Oospores.)
> *Plasmopara Kellermani* (Ell. et Halst.) Swingle ex Saccardo, Syll. Fung. **9**: 342. 1891. (Oospores.)
> *Basidiophora Kellermanii* (Ellis & Halsted) Wilson in Bul. Torrey Bot. Club **34**: 394. 1907. (Oospores.)

I believe *Basidiophora Kellermanii* (Swingle ex Sacc.) Wilson most nearly conforms with the Botanical Rules. Since *Peronospora Kellermanni* Ellis and Halsted is a *nomen nudum,* it has no standing. Although Swingle failed to assign a name to the fungus, he discussed its similarity to *"P. entospora,"* cited *P. "Kellermani"* as a synonym, mentioned finding oospores, and stated the host. Saccardo's description is obviously based on Swingle's discussion. Nevertheless, Saccardo was the first to validly publish a name for this downy mildew on *Iva xanthifolia* Nutt. If a shorter citation is desired, *B. Kellermanii* (Sacc.) Wilson is in conformity with the Rules. Saccardo, being the validly publishing author, must not be omitted.

2. **Bremia ganglioniformis** (Casp.) n. comb.—Usually cited as
   B. Lactucae Regel.

Bremia Lactucae Regel in Bot. Zeit. **1**: 666. T. 3B, fig. 1. 1843.

Botrytis ganglioniformis Berk. in Jour. Hort. Soc. Lond. **1**: 31. T. 4, fig. 25. 1846.

Peronospora ganglioniformis (Berk.) Tulasne in Compt. Rend. Acad. Sci. **38**: 1103. 1854. (Oospores observed; inadequately described.)

Peronospora ganglioniformis Tul. ex Caspary in Monatsb. K. Preuss. Akad. Wiss. **1855**: 330. 1855. (Reference made to both Berkeley and Tulasne.)

Peronospora gangliformis Berk. ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 106. T. 8, fig. 1–3. 1863. (Oospores.)

Bremia Lactucae Regel ex Schroeter in Cohn, Krypt. Fl. Schles. **3** (1): 239. 1886. (Oospores.)

Regel, in originally describing and illustrating the fungus, knew of only the imperfect stage. Although Tulasne was the first to observe the oospores, he stated merely that they were smooth. Since the fungus cannot be identified on this character alone, his combination must be considered a *nomen nudum*. Caspary, by referring to both Tulasne's paper and Berkeley's, fulfilled the requirements for valid publication.

Subsequent to Regel, Schroeter was the first to use the generic name *Bremia* again. He described the oospores in his generic description; the genus should therefore be cited *Bremia* Regel ex Schroeter, or, for brevity, *Bremia* Schroeter.

The above is one example of the type of changes that must be made if one applies Article 57 to the Phycomycetes. This case presents a strong argument in favor of Bisby's proposed change in Article 57. *Bremia Lactucae* Regel, a binomial well established in mycological and phytopathological literature, would be preserved and the new combination would not be necessary. Furthermore, it seems illogical to base the binomial on the perfect stage, when the fungus cannot be positively identified if only that stage is present. The imperfect stage, on the other hand, is diagnostic, and it alone serves to readily identify the fungus.

3. *Peronospora alta* Farlow—Usually cited as *Per. alta* Fuckel.

Peronospora alta Fuckel, Fungi rhen. No. 39. 1863. (Also Hedw. **2**: 133. 1863.)

Peronospora alta Fuckel ex Farlow in Bot. Gaz. **8**: 328. 1883. (Oospores.)

According to European workers, the oospores of *Peronospora alta* are rarely observed (7).   Farlow, in 1876 (5), was apparently the first to observe them and stated that they were especially abundant.   However, since Farlow incorrectly identified the fungus as *Per. effusa* in this work, the reference given above for oospores is to his later paper in which the name *Per. alta* is used.

4. *Peronospora Plantaginis* Burrill apud Underwood emend.— Usually cited as *Per. Plantaginis* Burrill.

Peronospora Plantaginis Burrill apud Underwood in Bul. Torrey Bot. Club **24**: 83. 1897.
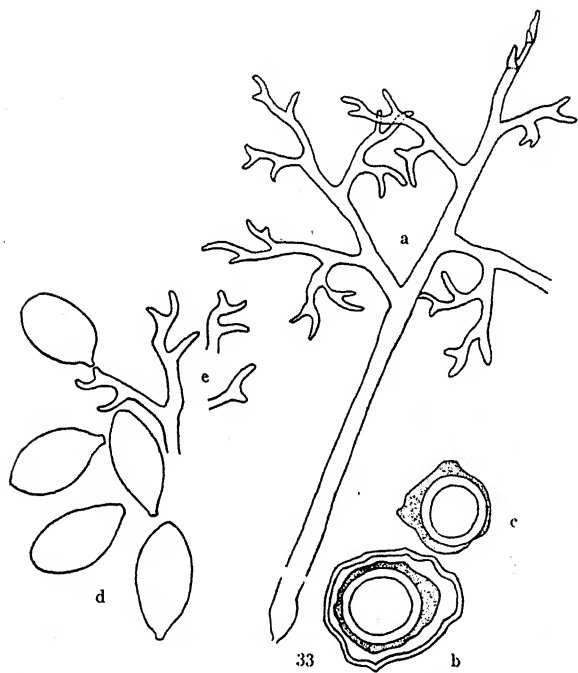
FIG. 1. *Peronospora Plantaginis.* a, d, e, on *Plantago aristata;* b, c, on *Plantago Purshii.*

Foliicolous; infected area light brown, extending the width of the leaf, appearing unoccupied below due to the sparseness of the mat and the pubescence of the host; conidiophores hypophyllous, aseptate, hyaline, emerging singly or in groups of 2–5 from the stomata, 4–6 times monopodially branched, $300–790 \times 7.5–12 \mu$; crown 1/3 or less the total height, main branches straight or nearly

so; ultimate branches acute, stout, unequal, curved, 4–15 μ long, the axial longer, occasionally sigmoid; conidia light brown to violaceous, elliptical to ellipsoid, somewhat pointed apically, distinctly pointed basally, often with a basal projection, 31–43 × 15–23 μ (Mean 34.37 × 19.21 μ); oogonia 38–60 μ diam., wall approximately 2.5 μ thick, dark yellow-brown; oospores 30–54 μ diam., exospore at most light yellow-brown, often unilaterally thickened up to 7 μ, externally uneven; endospore 2–4 μ thick, hyaline (FIG. 1).

On Plantaginaceae: Oospores and conidia on: *Plantago Purshii* R. & S. (Collected June 21, 1943. Madison, Dane Co., Wisc. H. C. Greene. Specimen deposited in the Cryptogamic Herbarium of the University of Wisconsin.) Conidia on: *Plantago aristata* Michx.

Range: Ala. to N. Car. and northward to southern Wisc.

As pointed out by Wilson (11), both *Peronospora Plantaginis* and *Per. alta* occur on members of the Plantaginaceae. The conidiophores of *Per. Plantaginis* exhibit a more nearly monopodial type of branching and are stouter; the main branches are straighter, and the ultimate branches are shorter and stouter. The conidia of *Per. Plantaginis* are somewhat larger (not smaller, as Wilson (11) states; see original description), are distinctly pointed basally and somewhat so apically. The oogonia of the two species are also distinct, those of *Per. alta* having thin, hyaline walls, while those of *Per. Plantaginis* have thick, dark yellow walls. *Per. Plantagnis* is therefore placed in de Bary's section Parasiticae, while *Per. alta* belongs in section Effusae.

The identity of the oospores noted by Wilson (11) in *Plantago pusilla* Nutt. remains unsettled, since they are much larger than those of either *Per. alta* or *Per. Plantaginis*. Their diameter, as reported by Wilson ("60–95 μ across"), is above the size usually encountered in members of the Peronosporaceae (4).

5. *Peronospora candida* de Bary—Usually cited as *Per. candida* Fuckel.

Peronospora candida Fuckel, Fungi rhen. No. 38. 1863. (Also Hedw. **2**: 132. 1863.)

Peronospora candida Fuckel ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 120. 1863. (Oospores.)

6. *Peronospora Alsinearum* de Bary—Usually cited as *Per. Alsinearum* Caspary.

> *Peronospora Alsinearum* Caspary in Monatsb. K. Preuss. Akad. Wiss. **1855**: 330. 1855.
> *Peronospora Alsinearum* Caspary ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 113. T. 8, fig. 9–18. 1863. (Oospores.)

7. *Peronospora effusa* de Bary—Usually cited as *Per. effusa;* the author citation varies.

> *Botrytis effusa* Grev., Fl. Edin. p. 468. 1824. (Pre-Friesian.)
> *Botrytis farinosa* Fries, Sys. Myc. **3**: 404. 1832.
> *Botrytis effusa* Grév. ex Desmazières in Ann. des Sci. Nat., Ser. II, **8**: 5. T. 1, fig. 1. 1837.
> *Perenospora farinosa* Fr., Sum. Veg. Scand. p. 493. 1849.
> *Peronospora effusa* Ces. in Rabenh., Herb. Myc., ed. I, No. 1880. 1854. (Also in Bot. Zeit. **12**: 190. 1854.)
> *Peronospora effusa* (Grev.) Tulasne in Compt. Rend. Acad. Sci. **38**: 1103. 1854. (Oospores; but inadequately described. Nomen nudum.)
> *Peronospora effusa* Grev. ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 115. T. 8, fig. 7; T. 13, fig. 11. 1863. (Oospores.)

Here is an instance where the adoption of Bisby's proposal would defeat "established custom," as the binomial *Peronospora effusa* de Bary would have to be replaced with *Per. farinosa* (Fries) Fries, a combination evidently overlooked by both Keissler and Wilson (**11**). Following Bisby's proposal, even if one recognizes several species of downy mildews on chenopodiaceous hosts, the epithet "farinosa" would have to be maintained for one of them, since that name was employed by Fries in his *Systema*.

8. *Peronospora Ficariae* de Bary—Usually cited as *Per. Ficariae* Tulasne.

> *Peronospora Ficariae* Tulasne in Compt. Rend. Acad. Sci. **38**: 1103. 1854. (Oospores observed; inadequately described. Nomen nudum.)
> *Peronospora Ficariae* Tulasne ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 117. 1863. (Oospores.)

Mere mention of the occurrence of oospores does not constitute a description of the fungus. Tulasne was the first to find oospores of this and other species of *Peronospora*. He briefly characterized the oospores of seven of them, but did not write descriptions. He failed to indicate the hosts and he did not refer to descriptions by

other authors. I see no alternative, therefore, but to consider Tulasne's binomials *nomina nuda*.

9. *Peronospora grisea* de Bary—Usually cited as *Per. grisea* (Unger) Unger.

> *Botrytis grisea* Unger, Exantheme d. Pfl. p. 172. 1833.
> *Peronospora grisea* Ung. in Bot. Zeit. **5**: 315. 1847.
> *Peronospora grisea* Unger ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 119. T. 13, fig. 12. 1863. (Oospores.)

10. *Peronospora obovata* de Bary—Usually cited as *Per. obovata* Bonorden.

> *Peronospora obovata* Bon. in Rabenh., Fungi europ. II, No. 289. 1860. (Also Bot. Zeit. **19**: 104. 1861.)
> *Peronospora obovata* Bonorden in Rabenh. ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 121. 1863. (Oospores.)

11. *Peronospora parasitica* de Bary—Usually cited as *Per. parasitica* (Pers.) Tul.

> *Botrytis parasitica* Persoon, Obs. Myc. **1**: 96. T. 5, fig. 6. 1796. (Pre-Friesian.)
> *Botrytis parasitica* Pers. ex Fries, Sys. Myc. **3**: 404. 1832.
> *Perenospora parasitica* Fr., Sum. Veg. Scand. p. 493. 1849.
> *Peronospora parasitica* (Pers.) Tulasne in Compt. Rend. Acad. Sci. **38**: 1103. 1854. (Oospores observed; inadequately described.)
> *Peronospora parasitica* Pers. ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 117. 1863. (Oospores.)

According to the present wording of Article 57, the proper citation is simply *Peronospora parasitica* de Bary. If Bisby's proposal is followed, the citation becomes *Per. parasitica* (Pers. ex Fr.) Fr. The volume in which Fries published this combination, and others, evidently has long been overlooked by taxonomists of the Peronosporaceae.

12. *Peronospora Potentillae* Farlow—Usually cited as *Per. Potentillae* de Bary.

> *Peronospora Potentillae* de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 124. 1863.
> *Peronospora Potentillae* de Bary ex Farlow in Bot. Gaz. **8**: 314. 1883. (Oospores.)

13. *Peronospora Schleideniana* Cornu—Usually cited as *Per. destructor;* the author citation varies.

Botrytis destructor Berk. in Ann. Mag. Nat. Hist. I, **6**: 436. T. 13, fig.
  23. 1841.
Peronospora Schleideni Ung. in Bot. Zeit. **5**: 315. 1847.
Perenospora destructor Fr., Sum. Veg. Scand. p. 493. 1849.
Peronospora Schleideniana Unger ex de Bary in Ann. des Sci. Nat., Ser.
  IV, **20**: 122. 1863.
Peronospora Schleideniana Unger ex Cornu in Bul. Soc. Bot. France
  25: 298. 1878. (Oospores.)

The nomenclature of the organism causing downy mildew of
onion has been discussed by several workers, including Yarwood
(**12**), Wakefield and Moore (**10**), Gäumann (**7**), and Wilson (**11**).
Wakefield and Moore, in attempting to follow the present wording
of Art. 57, credit G. W. Smith with first finding the oospores of the
fungus; they evidently overlooked Cornu's earlier description.
Since Cornu is the validating author, the spelling used by him for
the specific epithet is correct, rather than Unger's original spelling.
If Bisby's proposal is followed, the binomial now in common use is
the proper one, the correct author citation being *Per. destructor*
(Berk.) Fries.

14. *Peronospora Urticae* de Bary—Usually cited as *Per. deBaryi*
    Salm. & Ware.

  Peronospora Urticae (Lib.) de Bary in Ann. des Sci. Nat., Ser. IV, **20**:
    117. 1863. (Oospores.) (Synon. exclusis.)
  Peronospora deBaryi Salm. & Ware in Trans. Brit. Mycol. Soc. **14**: 47;
    figs. 1–6. 1929. (Oospores.)

15. *Pseudoperonospora Urticae* Salm. & Ware—Usually cited as
    Pseudoper. Urticae (Lib.) Salm. & Ware.

  Botrytis Urticae Lib. ex Berk. in Jour. Hort. Soc. Lond. **1**: 31. 1846.
    (Nomen nudum.)
  Botrytis Urticae Lib. ex Berk. & Broome in Ann. Mag. Nat. Hist.,
    Ser. II, **7**: 100. 1851.
  Pseudoperonospora Urticae (Lib.) Salm. & Ware in Trans. Brit. Mycol.
    Soc. **14**: 47. 1929. (Oospores.)

In a recent paper (**9**) I discussed the history of the two downy
mildew species occurring on *Urtica* spp. and pointed out that it is
Peronospora deBaryi Salm. and Ware which occurs in the U. S.
In that paper the nomenclature employed by Salmon and Ware was
used. If Bisby's proposal is followed, the nomenclature employed
by Salmon and Ware is essentially correct. I should prefer, how-
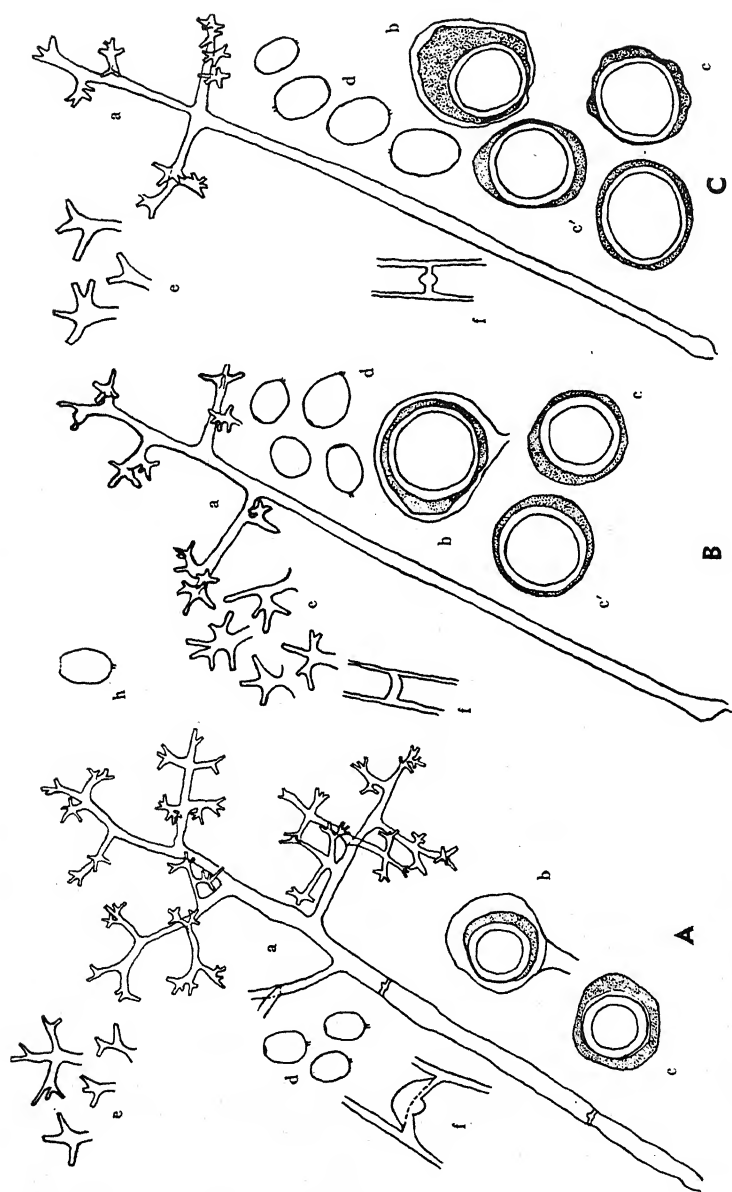
Fig. 2. A. Plasmopara Acalyphae on Acalypha virginica. B. Plasmopara ribicola on Ribes triste. C. Plasmopara Viburni on Viburnum Opulus var. americana.

ever, the citation *Pseudoperonospora Urticae* (Berk. & Broome) Salm. & Ware, since Berkeley and Broome must be considered the first to have validly published a binomial for the downy mildew occurring on *Urtica* and producing apically papillate, poroid conidia.

If Article 57, as now worded, is followed, the *Peronospora* becomes *Per. Urticae* de Bary, and the *Pseudoperonospora* becomes *Pseudoper. Urticae* Salm. & Ware. No other author should be be cited for either.

16. *Plasmopara Acalyphae* Wilson emend.—Usually cited as *Plas. Acalyphae* Wilson.

Plasmopara Acalyphae Wilson in Mycologia **10**: 169. 1918.

Foliicolous; infected area small, up to 1.0 cm. across, irregular in shape, at first chlorotic, becoming light brown, finally dark brown with a reddish margin; white, usually sparse, mat below; conidiophores hypophyllous, septate, hyaline, emerging from the stomata singly or in groups of 2–3, monopodially branched 4–5 (6) times, 280–570 × 5–10 μ; crown $\frac{1}{3}$ to $\frac{1}{2}$ the total height, loosely branched, primary branches 4–7 in number, straight, arising at acute to right angles; ultimate branches 2–5 in number, slightly conic, straight, narrow, truncate, very short, mostly 3–5 μ, a few up to 12 μ long; conidia hyaline, contents occasionally chlorinous, broadly ovate to subglobose, basally pedicellate, apically indistinctly poroid, 11–16 × 9–14 μ (Mean 13.91 × 11.10 μ); oogonia 33–44 μ diam., wall thin, yellowish; oospores small, 27–38 μ diam., exospore light yellowish, externally smooth, often thickened unilaterally up to 5 μ; endospore hyaline, 3–4 μ thick (FIG. 2, A).

On Euphorbiaceae: Oospores and conidia on: *Acalypha virginica* L. (Collected Oct. 14, 1926. Blue River, Grant Co., Wisc. J. J. Davis. Specimen deposited in the Cryptogamic Herbarium of the University of Wisconsin.)

Range: Wisconsin.

This is believed to be the first description of the oospores.

17. *Plasmopara Geranii* (Farlow) Berlese & De Toni—Usually cited as *Plas. Geranii* (Peck) Berlese & De Toni.

Peronospora Geranii Peck in Rept. N. Y. State Mus. Nat. Hist. **28**: 63. 1876.
Peronospora Geranii Peck ex Farlow in Bot. Gaz. **8**: 311. 1883. (Oospores.)

Plasmopara Geranii (Peck) Berl. et De Ton. in Sacc., Syll. Fung. **7** :
242. 1888. (Oospores.)

18. **Plasmopara Hepaticae** (Caspary) n. comb.—Usually cited
as *Plas. pygmaea* (Unger) Schroeter.

Botrytis pygmaea Unger, Exantheme d. Pfl. p. 172. 1833.
Peronospora pygmaea Ung. in Bot. Zeit. **5** : 315. T. 6, fig. 8. 1847.
Peronospora Hepaticae Caspary in Monatsb. K. Preuss. Akad. Wiss.
1855 : 329. T. 6, figs. 22–26. 1855. (Oospores.)
Plasmopara pygmaea (Unger) Schroeter in Cohn, Krypt. Fl. Schles.
3 (1) : 239. 1886. (Oospores.)

Caspary was the first to describe the oospores of this species.
Both "acrospores" (conidia) and "sporangia" (oogonia) were de-
scribed and figured for his *Peronospora Hepaticae*. According to
the present wording of Article 57, *Plasmopara Hepaticae* (Casp.)
n. comb. is correct, rather than *Plas. pygmaea* (Ung.) Schroet.,
which would be correct according to Bisby's proposal and which is
in common use.

19. *Plasmopara Illinoensis* Davis—Usually cited as *Plas. Illinoensis*
(Farlow) Davis.

Peronospora Illinoensis Farlow in Bot. Gaz. **8** : 332. 1883.
Plasmopara illinoensis (Farl.) Davis in Trans. Wis. Acad. Sci. **21** : 280.
1924. (Oospores.)

20. *Plasmopara nivea* (de Bary) Schroeter—Usually cited as *Plas.
nivea* (Unger) Schroeter.

Botrytis nivea Martius ex Unger, Exantheme d. Pfl. p. 171. T. 2, fig.
14. 1833. (Not *B. nivea* Martius, Fl. Erlang. 1817.)
Peronospora nivea Ung. in Bot. Zeit. **5** : 314. 1847. (Pro parte.)
Peronospora nivea Unger ex de Bary in Ann. des Sci. Nat., Ser. IV, **20** :
105. T. 4. 1863. (Oospores.)
Plasmopara nivea (Unger) Schroeter in Cohn, Krypt. Fl. Schles. **3**
(1) : 237. 1886. (Oospores.)

21. *Plasmopara ribicola* Davis emend.—Usually cited as *Plas.
ribicola* (Schroeter) Schroeter.

Peronospora ribicola Schroeter in Jahresber. d. Schles. Gesellsch. Vaterl.
Kult. **61** : 179. 1883. (Fide Schroeter, 1886.)
Plasmopara ribicola (Schroeter) Schroeter in Cohn, Krypt. Fl. Schles.
3 (1) : 238. 1886.
Plasmopara ribicola Schroet. ex Davis in Trans. Wis. Acad. Sci. **18** : 94,
251. 1915. (Oospores.)

In his 1886 publication, Schroeter gives the following reference as the place of publication of his *Peronospora ribicola:* "Jahresber. d. Schles. Gesellsch. f. 1883.   S. 179."   Examination of this publication disclosed that the cited page is included in an article by Schroeter in which four new species of Peziza are described on "S. 179."   No mention of a downy mildew could be found anywhere in the article, or in the entire volume.

Davis first described the oospores of *Plasmopara ribicola,* but failed to mention the hosts or the conidial stage, or to give any reference to a previous description.   He stated that the oogonial wall is symmetrically thickened on two opposite sides.   Actually it is the exospore wall that is so thickened, not the oogonial wall.   Such lateral thickening is characteristic of the oospores of several species of *Plasmopara*.   Since the description of the perfect stage, as published by Davis, was slightly inaccurate, and since a combined description of the oospores and the conidia of this fungus has not been published, a complete description is given below.

Foliicolous; infected area angular, limited by the veins, tan, gray-brown to dark brown or often reddish above; white, thin, loose mat below; conidiophores hypophyllous, septate, hyaline, fasciculate, 2–10 emerging from a stoma, (2) 3–5 times monopodially branched, 160–500 × 6–9 μ, base often bulbous, up to 11 μ; crown ⅓ to at most ½ the total height, main branches 2–6 in number, arising at approximately right angles, straight; ultimate branches 2–5 in number, narrowly conic, straight to slightly tortuous, narrowly truncate, mostly 4–14 μ long; conidia hyaline, broadly ellipsoid to subglobose, basal pedicel short, often indistinct; apically flatly poroid, variable in size, 11–32 × 10–22 μ (Mean 16.97 × 14.10 μ); oogonia 29–43 μ diam. wall thin, light yellow brown; oospores 26–40 μ diam., exospore hyaline when expressed, externally smooth, often thickened unilaterally or on two opposite sides up to 9 μ; endospore hyaline, 2–3 μ thick (FIG. 2, B).

On Saxifragaceae: Oospores and conidia on *Ribes Cynosbati* L., *R. gracile* Michx., *R. oxycanthoides* L., *R. prostratum* L'Her., and *R. triste* Pall. (Oospores occur in the following specimen which has received wide distribution: Fungi Columbiani No. 1753. On *Ribes rubrum subglandulosum* (= *R. triste* Pall.), Vilas Co., Wisc., July 29, 1902, J. J. Davis.)

Range: W. Va., westward to Wash.; also Europe.

23. *Plasmopara Viburni* Davis—Usually cited as *Plas. Viburni* Peck.

> *Plasmopara Viburni* Peck in Rept. N. Y. State Mus. Nat. His. **43**: 28. 1890.
>
> *Plasmopara viburni* Pk. ex Davis in Trans. Wis. Acad. Sci. **18**: 101. 1915. (Oospores.)

In describing the perfect stage of this species, Davis again characterized the oogonial wall as "often irregularly thickened." As in *Plasmopara ribicola,* I consider the exospore wall to be the structure laterally thickened. Since Davis mentioned the host and the occurrence of conidia, his description is accepted as valid. A complete description, however, is given below.

Foliicolous; infected areas angular, often located along the main veins and limited by the smaller veins, coalescing into large areas, at first watersoaked, becoming dark brown above; white, sparse, thin mat below; conidiophores hypophyllous, septate, hyaline or with light yellowish contents, up to 8 emerging from a stoma, 2–4 times monopodially branched, 120–440 × 4–9 μ, slightly bulbous basally; crown ⅓ to at most ½ the total height, main branches 2–5 in number, arising at right angles, straight or slightly tortuous, truncate, 4–17 μ long; conidia hyaline or with yellowish contents, broadly ovate, basally pedicellate, indistinctly poroid apically, variable in size, 13–34 × 11–23 μ (Mean 19.80 × 14.82 μ); oogonia 34–46 μ diam., wall thin, hyaline to light yellow; oospores 32–38 μ diam., exospore yellowish, usually appearing thin, slightly wrinkled externally, thickened laterally to 4.5 μ (occasionally to 10 μ); endospore hyaline, 3–4 μ thick (FIG. 2, C).

On Caprifoliaceae: Oospores and conidia on *Viburnum Opulus* L. var. *americanum* (Mill.) Ait. (Oospores occur in the following specimen which has received wide distribution: Fungi Columbiani No. 4280. On *Viburnum Opulus* L. var. *americanum* (Mill.) Ait. Wausaukee, Wisc. Aug. 23, 1913. J. J. Davis.)

Range: N. Y. westward to Wisc., southward to Ala.

24. *Plasmopara viticola* (de Bary) Berl. & De Toni—Usually cited as *Plas. viticola* (Berk. & Curt.) Berl. & De Toni.

> *Botrytis viticola* B. & C. ex Berkeley in Jour. Hor. Soc. Lond. **6**: 289. 1851. (Nomen nudum.)
>
> *Peronospora viticola* (Berkl. et Curtis) Caspary in Monatsb. K. Preuss. Akad. **1855**: 331. 1855. (Nomen nudum.)

Peronospora viticola (Berk. et Curt.) de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 125. 1863. (Oospores.)

Plasmopara viticola (Berk. et Curt.) Berl. et De Ton. in Sacc., Syll. Fung. **7**: 239. 1888.

Since Berkeley did not publish a description, it would seem to me that the correct citation is *Plas. viticola* (de Bary) Berl. & de Toni, both according to Article 57 and to Bisby's proposal concerning Art. 57, referred to above.

The examples above show that, regardless of whether the present wording of Article 57 or Bisby's proposal is followed, some changes in the binomials and author citations are necessary for certain species of the Peronosporaceae. Fewer changes in binomials would have to be made if Bisby's proposal were accepted than if Article 57 is strictly applied. Regardless of which procedure is followed, author citations in common use for several species are incorrect.

I am in favor of changing the wording of Article 57 so that its principle will not apply to the Phycomycetes. But, until that change is actually and validly made, the only justifiable procedure is to consider that Article 57 does apply to the Phycomycetes.

Problems relating to fungus nomenclature should be thoroughly discussed in the time remaining so that definite proposals can be presented to the Congress to be held in Stockholm in 1950. So that final decisions may be reached at that time, serious thought should be given to changing the provisions of Article 74, which prescribes the method by which the Rules may be altered. Considering the long intervals of time that have elapsed between previous congresses, the provisions of this article actually defeat the very purpose of the Rules, namely stability in nomenclature.

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EXPLANATION OF FIGURES

All figures drawn with the aid of a camera lucida. a, Conidiophore × 275. b, Oogonium with enclosed oospore; drawn in optical sectional view × 325. c, Oospore(s). The exospore has been stippled in all oospore and oogonial drawings as an aid to distinguishing the various structures. Oospores drawn as they appear in the oogonium are indicated by the letter "c"; expressed oospores, by "c'" × 325. d, Conidia × 325. e, Ultimate branches × 325. f, Septation × 900. h, Germinated conidium × 325.

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A NEW SPECIES OF ACHLYA

Helen Simpson Reischer

(with 5 figures)

In the spring of 1947 a series of collections of damp soil were made in Palisades Interstate Park, New Jersey, for the purpose of obtaining material for a comparative physiological study of the Saprolegniaceae. One of these collections proved to contain an *Achlya* which apparently has not previously been described, and for which the following name is proposed:

Achlya Sparrowii [1] sp. nov.

Mycelium in semine *Cannabis sativae* densum; cultura usque ad 1.5 cm. diam., hyphis primariis basi 60–80 μ diam. Sporangia rara, proliferantia ut in *Achlya* soleat. Exitus zoosporarum typicus generis; sporae hibernantes ca. 11 μ diam. Gemmae non formatae. Oogonia globosa, numerosa, plerumque 57 ad 77 μ diam., in ramulis lateralibus lata, cum tunica crassa, omnino levia et non punctulata. Ova 1–11, fere 3 vel 4 in oogonio quoque, plurimum 28.6–33 μ diam., maturitate subcentrica. Antheridia 1–4 in omni oogonio, origine androgyna, numquam ramosa, ad oogonium acumine applicata.

Mycelial growth dense, reaching a diameter of approximately 1.5 cm. on hemp seed. Main hyphae about 60 to 80 μ wide at the base. Sporangia somewhat rare, those formed 220–400 μ by 26–35 μ. Secondary sporangia produced by cymose branching from below. Zoospore discharge as typical for the genus, the encysted zoospores about 11 μ in diameter. Gemmae not formed in our cultures. Oogonia spheric, abundant, 33 to 101 μ in diameter, usually 57 to 77 μ, borne on short lateral stalks from the main hyphae, occasionally terminal on the main hyphae. Oogonial wall smooth, unpitted except under the antheridia. Oospores spheric, 1 to 11 in an oogonium, usually 3 or 4, mostly 28.6 to 33 μ in diameter, at maturity slightly subcentric with a circle (as seen in optical cross section) of small oil droplets, slightly thicker on one side, surrounding the protoplasm. Oospore wall smooth, thick. Antheridia on all oogonia, 1–4 per oogonium, arising from the oogonial

[1] This species is named in honor of Professor F. K. Sparrow, Jr.

stalk near the neck of the oogonium, from the neck of the oogonium, or less often from its spherical surface. Antheridia simple, clavate, always applied by the tip, never laterally applied and never branching. Fertilization tubes visible.

Type locality: Palisades Interstate Park, New Jersey.

The description given above was drawn from unifungal cultures less than five days old, grown on hemp seed, which has become the standard substratum for members of the Saprolegniaceae, in charcoal-treated distilled water in petri dishes, at 20° C. *A. Sparrowii*, in these cultures, showed a marked resemblance to *A. racemosa* in its smooth oogonia, nearly centric oospores, and the general appearance of the androgynous antheridia. The oospores of *A. racemosa* are, however, sufficiently smaller to prevent confusion of the two species. Older cultures on hemp seed showed affinities to *A. polyandra*, in which the oospores are only slightly smaller than in our fungus, by an increase in the usual number of oospores in an oogonium and in the number and length of the antheridial branches. These hemp-seed cultures, though regularly washed, contained various unidentified bacterial contaminants. It seems reasonable to suppose that, among other factors, available food and oxygen vary considerably in different parts of the culture and in time.

In order to determine the degree of variation in the important sexual characters, which could be produced by changing the method of culture, *Achlya Sparrowii* was also grown in pure culture on (Difco) corn meal agar with 1 g./liter of yeast extract added and in pure culture in 20 ml. portions (in 250 cc. Erlenmeyer flasks) of a liquid medium consisting of 5 g./liter yellow corn meal (extracted by boiling for ½ hour and centrifuged clear before use) and 1 g./liter of yeast extract, at temperatures ranging from 10 to 30° C. at 5 degree intervals. The mycelial mats grown in the liquid medium were transferred to sterile charcoal-treated distilled water in petri dishes immediately after the formation of the first oogonial initials (after 3 to 5 days, depending upon the temperature). No growth occurred at 30° C. under any circumstances. Growth at 10° C. was quite slow, and the oogonia and oospores formed (on agar and hemp seed) frequently aborted. No further use, therefore, was made of these temperatures.

Temperature had no obvious effect upon the antheridia; the medium employed, and the age of the culture (in hemp-seed cultures only), affected the number, origin, size and shape, and the degree of branching of the antheridial stalks. In young cultures on hemp seed the antheridia were 1–4, usually 2, to an oogonium, a slight majority arising from the neck of the oogonium, nearly as many from the stalk just below the oogonial neck, a few from the spherical surface of the oogonium. No branching of the antheridial stalks was observed. Cultures on agar exhibited substantially the same characteristics. In hemp-seed cultures over five days old, however, slightly more of the antheridia arise from the oogonial stalk; the antheridial branches, usually about 25μ in length in the younger cultures and those on agar, become elongated loops up to 250μ in length, exclusive of the antheridium, and the antheridia, clavate in younger cultures, become tubular, with a diameter slightly less than that of the antheridial stalk. There may be, though rarely are, as many as eight antheridia applied to a single oogonium and, *very rarely*, the antheridial stalks may branch, though never the antheridia. Mats grown in the liquid medium described produce antheridia resembling those of the older hemp-seed cultures but showing even greater tendency to arise from the oogonial stalk. The author regards this as an indication that the change from an *A. racemosa* (as figured by Coker, 1923, pl. 31, f. 9) type of antheridial branch to an *A. polyandra* (as figured by Hildebrand, 1867, pl. 16, f. 7–11) type *may* be caused by the low concentration of nutrient materials in the older hemp-seed cultures. It is unfortunate that more attention has not been paid to the effect of such environmental factors upon taxonomically critical characteristics. The characters remaining constant throughout our cultures were the androgynous origin of the antheridia, the lack of branching in the antheridia themselves, and the terminal application of the antheridia to the oogonium. Since the branching of the antheridial stalks of *A. polyandra* is emphasized in all published descriptions of this species, and *A. polyandra* is figured with laterally applied antheridia (Hildebrand, 1867), these characters separate this closely related species from *A. Sparrowii*.

The diameter of the oogonium is closely correlated with the number and diameter of the oospores formed and is therefore of

minor importance as a separate character, varying from 33 μ (one oospore) to 101 μ (11 oospores), usually 57 to 77 μ (2 to 4 oospores) in the younger cultures on hemp seed.

Temperatures from 15 to 25° C. had no apparent effect on the number of oospores formed in the hemp-seed cultures. Three or four oospores per oogonium were usually produced in the younger cultures, 6 in the older cultures. In cultures on agar the oospore number did not vary with time but did vary slightly with temperature: mostly 2–3 oospores at 25° C., 3 at 20° C., and 3–4 at 15° C. Since a difference of 1 or 2 in the usual number of oospores has not been used to distinguish species, except where only one oospore is typically produced, a detailed statistical study was not considered necessary. Oogonia produced by mats grown on liquid media usually contained only 1 or 2 oospores, a probable result of lack of food materials, as similar results are obtained by placing agar discs containing mycelium in water at room temperature. It is noteworthy that the usual number of oospores produced does not approach the 10–15 typical of *A. polyandra*. In this respect our fungus resembles *A. racemosa* (described in Coker, 1923, as having usually 2–5 oospores) much more closely.

In order to ascertain whether a correlation existed between the number of oospores in an oogonium, and oospore diameter, 321 oospores from hemp-seed cultures grown at 15° C. were measured. The size of the samples taken was as follows: 22 oospores from oogonia containing 1 oospore; 50 oospores each from oogonia containing 2, 3, 4, 5, and 6 oospores; 30 from oogonia containing 7; 9, 7 and 3 oospores from oogonia containing respectively 8, 9 and 10 oospores. Half of the oospores in each of the larger samples were from cultures 5 days old, half from 8 day cultures. While the diameter of the oospores reaches extremes of range in oogonia containing only 1 oospore, no significant correlation of diameter with either number of oospores or age of culture was found. The combined results (the broken line in figure 1) give a typical distribution curve of oospore diameter for *A. Sparrowii* in hemp-seed culture. In determining the effect of temperature on oospore size, measurements were made, to prevent possible bias, only on oospores (one per oogonium) in oogonia containing 3 oospores (since singly produced oospores are apt to be aberrant, and the oospores of oogonia contain-

ing only two oospores are typically flattened spheres, the true diameter of which cannot be directly measured). Diameter did not vary clearly with temperature in the cultures on hemp seed, perhaps because so many other variables are present in such impure cultures. Measurements of 30 oospores each from hemp-seed cultures grown at 15, 20 and 25° C. fell substantially on the curve for the hemp-seed cultures in figure 1. Mats grown in a liquid medium did not
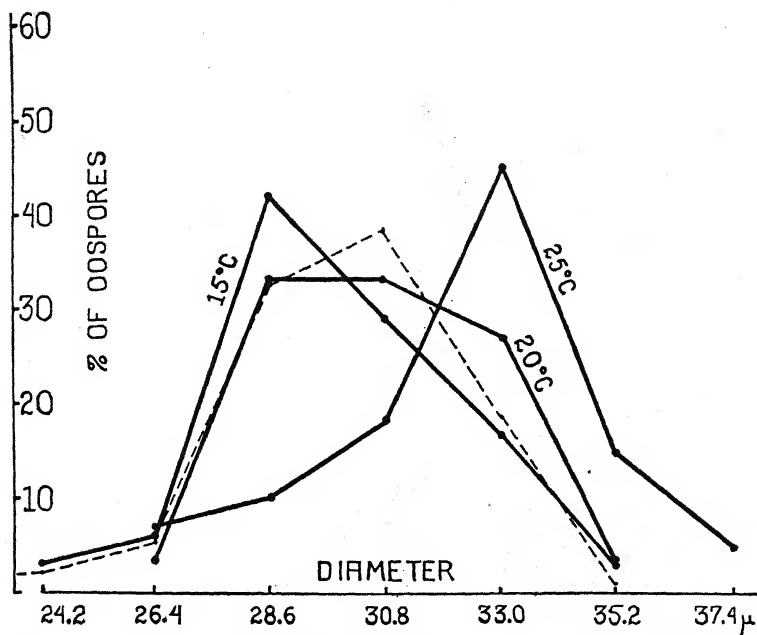
FIG. 1. The effect of temperature on oospore diameter in *Achlya Sparrowii*. Solid lines: cultures on agar grown at 15, 20 and 25° C. Broken line: hemp-seed cultures grown at 15° C.

produce oospores suitable for measurement. The variation found in cultures grown on agar is shown graphically in figure 1. The diameter of 35 oospores from cultures grown at 15° C., 30 from cultures grown at 20° C., 60 (in two samples of 30) from cultures grown at 25° C., was measured in units of 2.2 μ. The figures on the abscissa in figure 1 represent, then, the mean of a size class including 2.2 μ. The difference between the mean diameter of oospores produced at 15° C. (29.9 $\pm$ 0.380 μ) and 25° C. (32.3 $\pm$

$0.338\,\mu$) is statistically significant.   The oospores of *A. Sparrowii* are considerably larger than the "most about $22\,\mu$" (Coker, **1923**) of *A. racemosa;* no experimental treatment brought the average
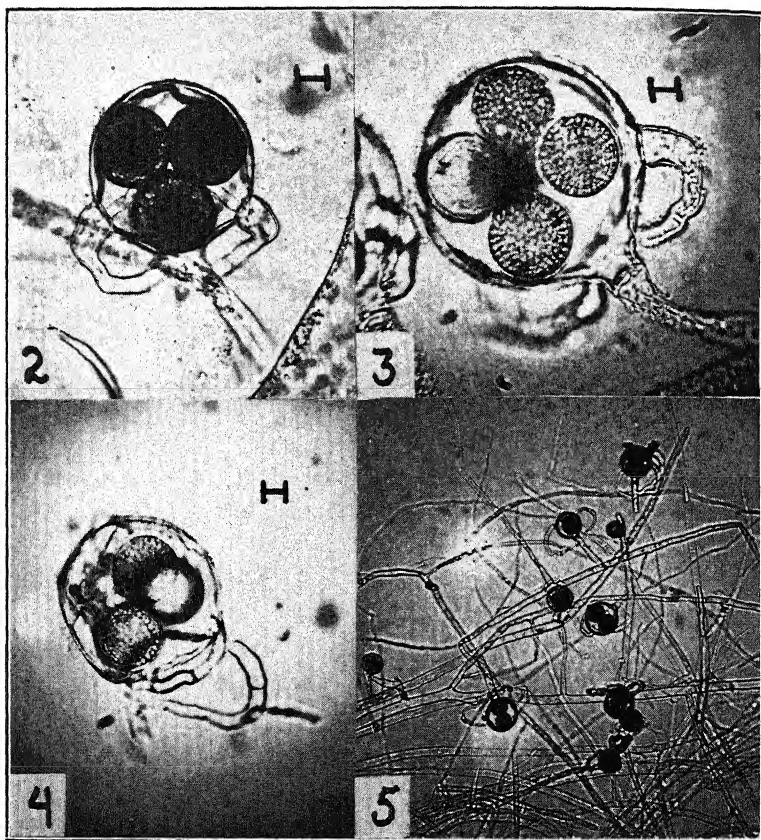
FIGS. 2–5.   *Achlya Sparrowii.*   The photomicrographs were taken with a Leica Makam using $6\times$ and $10\times$ oculars and a $44\times$ objective for figures 2–4, a $10\times$ objective for figure 5.   The lines drawn on figures 2–4 indicate the relative length of $10\,\mu$.   FIG. 2.   An oogonium with 3 oospores, antheridia arising from the neck of the oogonium and the oogonial stalk.   The fertilization tubes are visible. $\times\,400$.   FIG. 3.   An oogonium with 5 nearly mature oospores, an antheridium arising from the spherical surface of the oogonium near the neck. $\times\,400$.   FIG. 4.   An oogonium with 4 mature oospores, showing the slightly subcentric structure of the oospores at maturity. $\times\,325$. Figures 2–4 from hemp-seed cultures.   Fig. 5.   A group of hyphae from a mat grown on a liquid medium.   Note the reduction of oospore number to one or two, the long looping antheridial branches.   Approximately $\times\,60$.

oospore diameter as low as the 27 μ (Humphrey, 1892) of *A. polyandra*. Oospore diameter appears to be a relatively stable, and therefore extremely useful, taxonomic character.

SUMMARY

A new species of *Achlya* has been described under the name *Achlya Sparrowii*. Among the species of *Achlya* with centric or slightly subcentric oospores, androgynous antheridia and smooth oogonial walls there are only two, *A. racemosa* and *A. polyandra*, which closely resemble this fungus. *A. Sparrowii* may be distinguished from *A. racemosa* quite readily by its much larger oospores, from *A. polyandra* by its slightly larger, fewer oospores and the usually fewer, only rarely branched antheridial stalks with antheridia applied to the oogonia by the tip rather than laterally. The effect of growth in pure culture on liquid and solid media and of common laboratory temperatures on these characters has been described.

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TWO SPECIES REPRESENTING A NEW GENUS OF THE CHAETOMIACEAE

R. K. Benjamin [1]

(WITH 33 FIGURES)

In the course of a study of some coprophilous fungi, the author found what appears to be an undescribed ascomycete resembling in certain fundamental features fungi placed in the Chaetomiaceae. The first perithecia were found growing on goat dung and mass spore inoculations from these were made on potato dextrose agar and Blakeslee's No. 230 but on neither media were perithecia produced or anything other than sterile mycelia, even after two months. Transfers were made to dung agar and on this medium perithecia matured normally in less than three weeks and were identical to those which were found growing naturally on goat dung.

While attending the Chicago meetings of the Mycological Society of America in December 1947, the author mentioned to Professor G. W. Martin the isolates that he had been studying and Professor Martin indicated immediately that he had obtained a very similar fungus from a collection made in Peru in 1945. Professor Martin later very kindly sent cultures of his isolate for study. It was obvious from the first that the two organisms were congeneric but specifically distinct. These have been studied side by side on various types of media and their characteristics and differences noted. The characteristics of the perithecial walls, the evanescent asci and light-colored spores, the appendages surrounding the ostiole of the perithecium, and the manner in which the ascospores are discharged in the form of an elongate cirrus suggest a relationship to the genus *Chaetomium*. An examination of the monographs by Bainier (1), Palliser (4), and Chivers (2), and the more recent papers by Greathouse and Ames (3), Tschudy (5) and others has indicated that neither of these species could be as-

signed to this genus or any related genera in the family Chaetomiaceae. Because of their long necks and submerged habit of growth these two isolates are being designated here as representing new species of a new genus.

Lophotrichus gen. nov.[2]

Perithecia immersa vel semiimmersa, globosa, collo longo et angusto, fusca et opaca, ostiolata; pariete membranaceo et fragili, cui sunt appendiculae in similitudinem pilorum varie immutatorum redactae; ascis tenuibus, delicatis, subglobosis vel late clavatis cum stirpe brevi, evanidis, octosporis, qui paraphysibus carent; sporis unicellularibus, pallidis, pomi citrei formam referentibus.

Perithecia submerged or partially superficial, spherical and translucent when young, when mature globose with a long, narrow neck, dark and opaque, tip of neck pierced by an ostiole. Perithecial wall membranaceous, brittle, provided with appendages in the form of variously modified hairs. Mycelium mostly submerged, rarely superficial. Asci thin-walled, delicate, subglobose to broadly clavate and short stalked, very evanescent, 8-spored, paraphyses lacking. Spores single-celled, light-colored, lemonshaped.

Type species, *Lophotrichus ampullus*.

Lophotrichus ampullus sp. nov.   (FIGS. 1–16)

Perithecia in fimo agaris nata, nigra, globosa, 150–260 μ in diametro (vulgo 195 μ), immersa vel semiimmersa, quorum pars immersa mycelia fusca et rhizoidea, pars superior pilos laterales formam hypharum incoloratarum, septatarum et aëriarum habentes ferunt, tenuia, membranacea; collis plerumque singulis sed interdum binis, nigris, 130–760 μ longis vel etiam longioribus, 40–60 μ in diametro; pilis terminalibus, qui ostiolum cingunt, septatis, crassis, rectis sive inaequabiliter contortis, usque ad 1.6 mm. longis, 3.8–5.3 μ in diametro (vulgo 4.3 μ), parietes 0.57–1.52 μ crassos (vulgo 1.13 μ) habentibus, fuscis et quasi fumosis, plus minusve confertim incrustatis, cacumina curva sive circinata ferentibus; ascis subglobosis vel late clavatis cum stirpe brevi, incoloratis, 10–20 μ × 20–34 μ, evanidissimis, octosporis, qui paraphysibus carent; ascosporis, quae cum extruduntur cirrum usque ad 1.5 mm. longum saepius figurant, colorem cyprii cum glomerantur referentibus, hyalinis, pomi citrei formam habentibus, 5.3–7.6 μ × 6.8–10.6 μ (vulgo 5.97 μ × 8.75 μ), tenui pariete praeditis in cacuminibus, eo modo formatis ut utrubique germinent.

[2] The author is indebted to Professor Revilo Oliver, Classics Department, for the preparation of the Latin diagnoses.
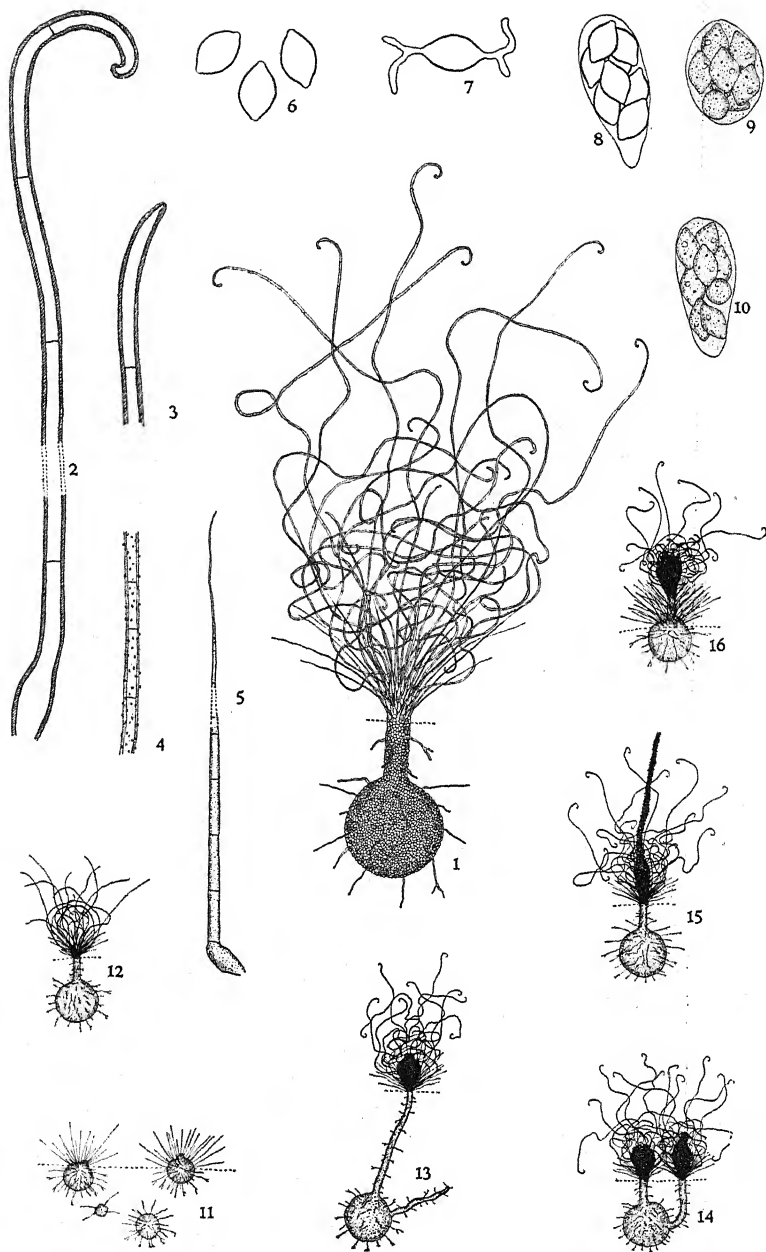
Figs. 1–16. *Lophotrichus ampullus.*

Mycelium on dung agar developing rapidly, remaining white, submerged, rarely with aerial hyphae; perithecia forming in 3–5 days, maturing in 2–3 weeks, black, globose, 150–260 μ in diameter (av. 195 μ), immersed or partially superficial, with dark, rhizoid-like mycelia on submerged parts, or lateral hairs in the form of colorless, septate, aerial hyphae on superficial portions; walls thin, membranaceous, necks usually one, occasionally two on a perithecium, black, 130–760 μ long or longer, more or less uniform in diameter, 40–60 μ; lateral hairs colorless, septate, acuminate, up to 150 μ long, 2–3 μ in diameter at base; ostiole surrounded by many long, septate, thick-walled terminal hairs, straight or irregularly contorted, up to 1.6 mm. long, 3.8–5.3 μ in diameter (av. 4.3 μ), walls 0.57–1.52 μ thick (av. 1.13 μ), dark, smoky in color, more or less densely encrusted, tips curved to circinate; asci subglobose to broadly clavate and short stalked, colorless, 10–20 μ × 20–34 μ, very evanescent, 8-spored, paraphyses lacking; ascospores extruded as a cirrus, frequently up to 1.5 mm. in length, bright copper colored in mass, hyaline, lemon-shaped, tips thin-walled, 5.3–7.6 μ × 6.8–10.6 μ (av. 5.97 μ × 8.75 μ), germinating at both ends.

Isolated from goat dung collected near Urbana, Illinois, November 1947. Herbarium material of the isolate representing the type deposited in the Mycological Collections, University of Illinois Herbarium (Mycological Collections No. 1763); the Mycological Collections of the Bureau of Plant Industry, Beltsville, Md.; Farlow Herbarium, Harvard University; and the Herbarium of the State University of Iowa, Iowa City.

Lophotrichus martinii sp. nov.[3]   (Figs. 17–33)

Perithecia in fimo agaris nata, nigra, globosa, 220–330 μ in diametro (vulgo 250 μ), immersa vel semiimmersa, quorum pars immersa mycelia fusca et rhizoidea, pars superior pilos laterales formam hypharum incoloratarum, septatarum et aëriarum habentes ferunt, tenuia, membranacea; collis plerumque singulis sed nonnunquam binis, trinis vel quaternis, nigris, 200–1000 μ longis vel etiam longioribus, 40–65 μ in diametro; pilis terminalibus, qui ostiolum cingunt, septatis, crassis, rectis sive curvis, plerumque 130–450 μ longis, quorum duo vel plures se usque ad 1.5 mm. solent porrigere, 3.8–6.1 μ in diametro (vulgo 5.2 μ), parietes 1.15–2.28 μ crassos (vulgo 1.9 μ) habentibus, fuscis et quasi fumosis, plus minusve confertim incrustatis, et cacumina recta sive curva ferentibus; ascis subglobosis vel late clavatis cum stirpe brevi, incoloratis, 11–17 μ × 20–36 μ, evanidissimis, octosporis, qui paraphysi-

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[3] Named in honor of Professor G. W. Martin, who very kindly supplied this isolate for study.
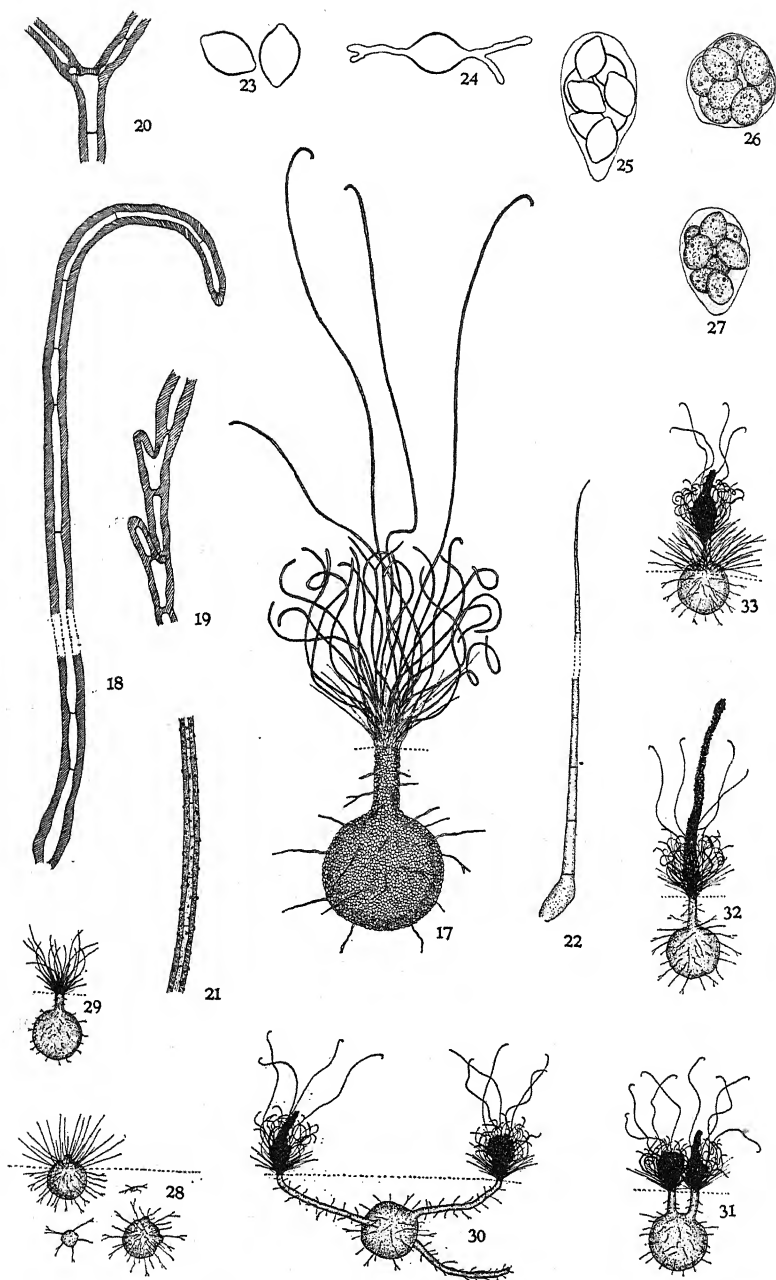
Figs. 17–33. *Lophotrichus martinii.*

bus carent; ascosporis, quae cum extruduntur cirrum usque ad 1.5 mm.
longum saepius figurant, colorem cyprii cum glomerantur referentibus, hya-
linis, pomi citrei formam habentibus, 5.3–6.8 μ × 7–9.9 μ (vulgo 6.4 μ × 8.9 μ),
tenui pariete praeditis in cacuminibus, eo modo formatis ut utrubique
germinent.

Mycelium on dung agar developing rapidly, remaining white,
submerged, rarely with aerial hyphae; perithecia forming in 3–5
days, maturing in 2–3 weeks, black, globose, 220–330 μ in diam-
eter (av. 250 μ), immersed or partially superficial, with dark,
rhizoid-like mycelia on submerged parts, or lateral hairs in the
form of colorless, septate, aerial hyphae on superficial portions;
walls thin, membranaceous, necks usually one, commonly two to
four on a perithecium, black, 200–1000 μ long or longer, more or
less uniform in diameter, 40–65 μ; lateral hairs colorless, septate,
acuminate, up to 150 μ long, 2–3 μ in diameter at base; ostiole sur-
rounded by many long, septate, thick-walled terminal hairs, straight
or curved, mostly 130–450 μ long with two or more commonly
reaching 1.5 mm. in length, 3.8–6.1 μ in diameter (av. 5.2 μ), walls
1.15–2.28 μ thick (av. 1.9 μ), dark, smoky in color, more or less
densely encrusted, tips straight or curved; asci subglobose to
broadly clavate and short stalked, colorless, 11–17 μ × 20–36 μ,
very evanescent, 8-spored, paraphyses lacking; ascospores extruded
as a cirrus frequently 1.5 mm. in length, bright copper colored in
mass, hyaline, lemon-shaped, tips thin-walled, 5.3–6.8 μ × 7–9.9 μ
(av. 6.4 μ × 8.9 μ), germinating at both ends.

Isolated from dung (rabbit?) collected near Talara, Peru,
September 1945, GWM No. 6290. Herbarium material of the iso-
late representing the type deposited in the Mycological Collections,
University of Illinois Herbarium (Mycological Collections No.
1762); the Mycological Collections of the Bureau of Plant In-
dustry, Beltsville, Md.; Farlow Herbarium, Harvard University;
and the Herbarium of the State University of Iowa, Iowa City.

While these species are similar in many respects their differences
are quite marked when the two are compared critically. Cultures
of *Lophotrichus martinii* with numerous ascocarps appear to be
distinctly darker when examined under low power magnification
due largely to the greater average size of the perithecia and the
much thicker walls of the terminal hairs.

When mature, a majority of the terminal hairs of *Lophotrichus
ampullus* are a millimeter or more in length. Some are highly con-
torted so that they form a loose tangle about the ostiole while many

others are nearly straight or only slightly curved. These hairs extend upward and outward above the substrate, and have tips which are usually curved to circinate, seldom straight. By the time the ascospores have been discharged into the terminal hairs the neck has become extremely brittle slightly below the ostiole so that the least disturbance frees the mass of hairs and spores for subsequent dispersal.

The terminal hairs of *L. martinii* are distinctly different from those of the former species. The majority of them are relatively short and straight-tipped or are slightly curved to contorted, and only a few extend to the greater length common for hairs of *L. ampullus*. The walls of the hairs of *L. martinii* are much thicker than those of *L. ampullus* and less uniform in thickness throughout the length of the hair. It is not uncommon to find branching of the terminal hairs in *L. martinii* while this phenomenon has not been observed in *L. ampullus*. Spore dispersal is effected as in the latter species.

The relative abundance of perithecia possessing more than one neck may be a useful characteristic to aid in the separation of these species. This phenomenon is common to both, but differs greatly in degree. Perithecia of *L. ampullus* may develop two necks, rarely more, but this occurs in a very small percentage of ascocarps. Perithecia of *L. martinii,* on the other hand, commonly develop two to four necks, and the number of such ascocarps may reach 25 per cent or more in any given culture. While all of the necks may reach the surface of the media, develop terminal hairs and function normally, some never reach the surface. The development of terminal hairs does not occur in either speices until the tip of the neck reaches the surface of the substrate, except occassionally in very old cultures, and in such cases the pattern of development is much modified. In rare instances branching of the neck has been observed, in which case the branch may develop terminal hairs also and function in spore dispersal.

The family Chaetomiaceae has been defined to include species which characteristically form ascocarps on the surface of the substrate. Species of *Lophotrichus* typically produce perithecia partially or entirely submerged with only the ostiole and terminal hairs exposed. The production of submerged or partially submerged

perithecia is a departure from the usual habit of species of the Chaetomiaceae. When, however, all characters are taken into consideration the author believes that *Lophotrichus* is best accommodated in the Chaetomiaceae.

Lophotrichus superficially resembles also such genera as *Melanospora* and *Ceratostomella* in that it possesses an elongate neck with conspicuous hairs around the ostiole.

On the basis of the characteristics of the asci it would seem that the Chaetomiaceae, and perhaps other fungi whose asci are so very evanescent, now generally included in the Pyrenomycetes, would be more logically placed if removed to the Plectomycetes. Certainly the Chaetomiaceae and the Sordariaceae have little in common when one considers the nature of the ascus, yet these families are most frequently placed near each other in the Sphaeriales.

SUMMARY

A new genus, *Lophotrichus,* including two species, *L. ampullus* and *L. martinii,* is described, which has certain characteristics of the Chaetomiaceae. The two described species of this genus produce perithecia which, when grown in culture, are partially or entirely submerged, possess long necks, and have elongate, thick-walled terminal hairs restricted to the area immediately around the ostiole. Ascus characteristics are essentially the same as in the genus *Chaetomium.* The more or less submerged habit of the perithecia and the typical long necks are departures from the usual concepts held for the Chaetomiaceae.

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EXPLANATION OF FIGURES *

Lophotrichus ampullus. Figs. 1–16

Fig. 1. Mature perithecium with cirrus of ascospores not shown. × 65. Fig. 2. Terminal hair with circinate tip. × 500. Fig. 3. Tip of terminal hair only slightly curved. × 500. Fig. 4. Portion of terminal hair showing encrustations. × 335. Fig. 5. Lateral hair. × 500. Fig. 6. Mature ascospores, in outline, showing thin end walls. × 835. Fig. 7. Germinating ascospore, in outline. × 835. Fig. 8. Outline of mature ascus just prior to breakdown of ascus wall. × 665. Figs. 9–10. Young asci showing variation in shape. × 665. Fig. 11. Young perithecia. × 25. Fig. 12. Perithecium before extrusion of ascospores. × 25. Figs. 13–14. Perithecia showing development of more than one neck. × 25. Figs. 15–16. Mature perithecia showing typical cirri of ascospores. × 25.

Lophotrichus martinii. Figs. 17–33

Fig. 17. Mature perithecium with cirrus of ascospores not shown. × 65. Fig. 18. Terminal hair showing thick wall and curved tip. × 500. Fig. 19. Portion of terminal hair showing development of short branches. × 500. Fig. 20. Portion of terminal hair showing dichotomous branching. × 500. Fig. 21. Portion of terminal hair showing encrustations. × 335. Fig. 22. Lateral hair. × 500. Fig. 23. Mature ascospores, in outline, showing thin end walls. × 835. Fig. 24. Germinating ascospore, in outline. × 835. Fig. 25. Outline of mature ascus just prior to breakdown of ascus wall. × 665. Figs. 26–27. Young asci showing variation in shape. × 665. Fig. 28. Young perithecia. × 25. Fig. 29. Young perithecium before extrusion of ascospores. × 25. Figs. 30–31. Perithecia showing development of more than one neck. × 25. Figs. 32–33. Mature perithecia showing typical cirri of ascospores. × 25.

* Dotted line with figures of perithecia indicates level of substrate.

NOTES AND BRIEF ARTICLES

TARGET SPOT OF COWPEA AND SOYBEAN. Recently Olive *et al.* (Phytopath. **35**: 822–831. 1945) described what appeared to be a new leaf-spot disease of cowpea and soybean. The causal organism was named *Helminthosporium vignae*. However, an earlier paper describing the disease and its causal agent has just come to the writer's attention. Kawamura (Fung. Nippon Fungological Soc. **1**: 14–20. 1931) found the fungus on cowpea in Japan and described it as *Cercospora vignicola* nov. sp. During the past year, Liu (Bot. Bull. Acad. Sinica **2**: 69–80. 1948) reported that the disease is a common one in China on cowpea and soybeans. He found that leaves and pods of the soybean are attacked by the fungus, and that the fungus may penetrate into the seeds and spot them or even prevent them from maturing. Liu prefers Kawamura's classification of the pathogen as a *Cercospora*.

The present writer remains of the opinion that this fungus should be classified as a species of *Helminthosporium* rather than *Cercospora*. It is true that if diseased leaves of cowpea and soybean are placed in a moist chamber most of the conidia which are produced will be long and narrow. But in nature the conidia are usually broader, typically less than ten times as long as broad. At maturity they are quite brown in color, and their walls and septa become characteristically thickened. Thus the fungus appears to fit better into the *Helminthosporium* concept. In view of these considerations, the writer proposes the following classification of the fungus: **Helminthosporium vignicola** (Kawamura) comb. nov. (Syn. *Cercospora vignicola* Kawamura; *Helminthosporium vignae* Olive).—Lindsay S. Olive, Louisiana State University.

PROPOSED FACSIMILE REPRINTS OF MYCOLOGICAL CLASSICS. Four works of great importance and daily usefulness to mycologists and others concerned with fungi have been selected for photo-offset reproduction:

Persoon, C. H.   Synopsis methodica fungorum $20.00
Fries, E.   Systema mycologicum (3 vols.) + Elenchus
    fungorum $50.00
Lindau, G., & Sydow, P.   Thesaurus litteraturae myco-
    logicae et lichenologicae.   (5 vols.) $90.00
Zahlbruckner, A.   Catalogus lichenum universalis.   (10
    vols.) $175.00

The first and second of these works are the points of departure
for the nomenclature of all groups of fungi except the myxomy-
cetes.   The third lists the literature of mycology and lichenology,
and on fungus diseases, to 1910, and is of great service in running
down references to work in these fields.   The fourth is the lichen-
ologists' equivalent of Saccardo.   For years all have been for the
majority of workers impossible to secure.

These works will be published only if the number of sales will
be great enough to support the project.   It is therefore requested
that *tentative* subscriptions, either personal or institutional, be
sent to the undersigned.—D. P. Rogers, New York Botanical
Garden, New York 58, N. Y.
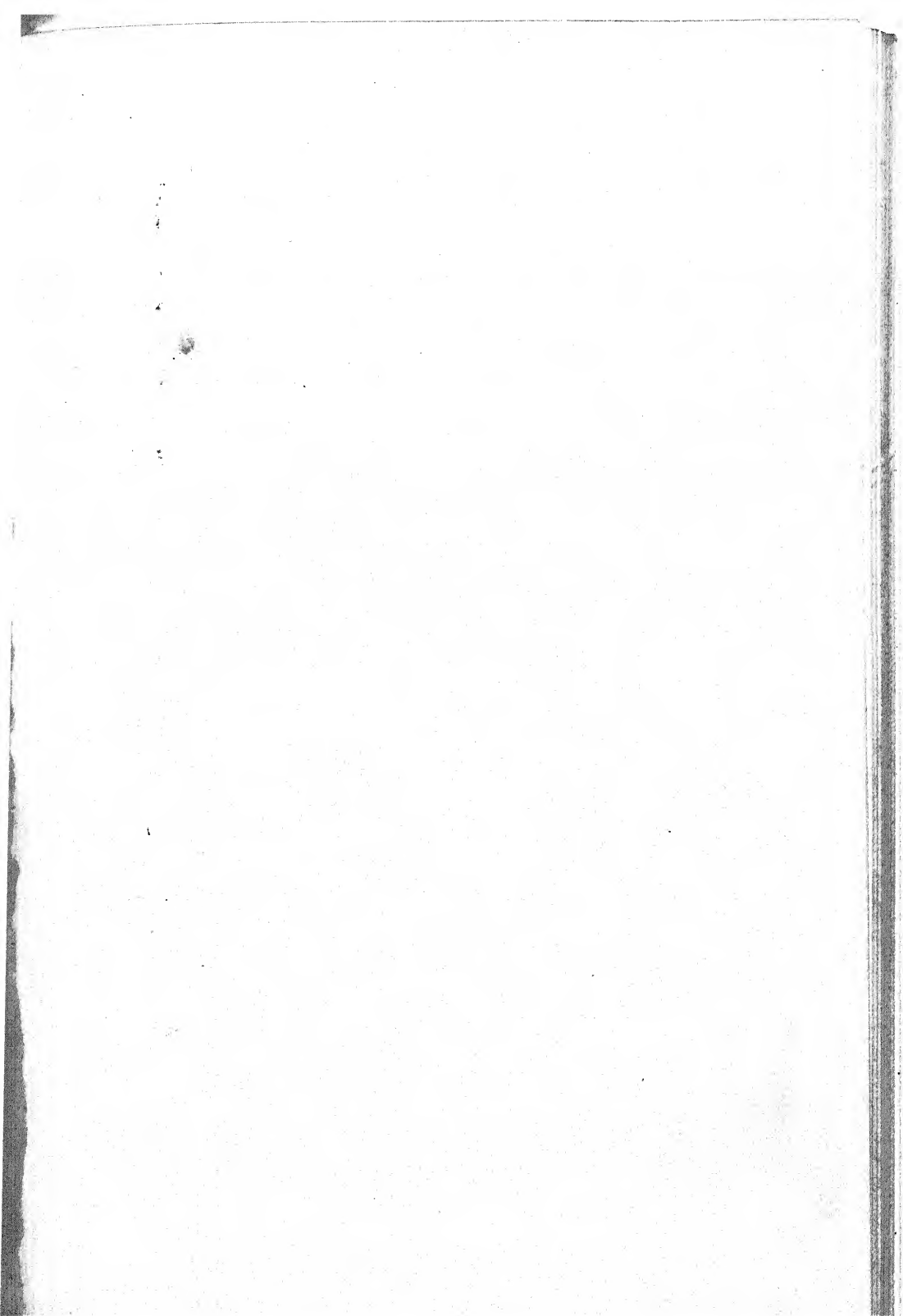
Sanford M. Zeller, 1885–1948

SANFORD MYRON ZELLER

October 19, 1884–November 4, 1948

HELEN M. GILKEY

Sanford Myron Zeller was born October 19, 1884 in Coldwater, Michigan. Like many another scientist son of a minister, he was born into a home in which culture and education were accepted as a matter of course, and earlier years of schooling naturally led to college.

His interest in science was aroused in high school by a teacher, Miss Burton, whose stimulating presentation and strict discipline in study methods always remained gratefully and affectionately in his memory. After high school he entered Lawrence College in Wisconsin, later transferring to Greenville College in Illinois, which granted him his Bachelor of Science degree in 1909. At the University of Washington in Seattle, where he later became instructor in botany, he obtained the degrees of Bachelor and Master of Arts.

A research fellowship in botany at Washington University, St. Louis, enabled him to receive a Ph.D. at that institution in 1917, where he remained as special investigator in dendropathology until his appointment in 1919 as assistant pathologist on the staff of the Oregon Agricultural Experiment Station at Corvallis. He remained for twenty-nine years of continuous service at this institution, his official title at the time of his death being Plant Pathologist and Professor of Plant Pathological Research.

During his years at the University of Washington and at the Missouri Botanical Garden, several summers were spent at Friday Harbor Biological Station on Puget Sound, where his main interest was in algae and fungi. That this interest crystallized on the side of fungi rather than algae, seems attributable to two circumstances —one, a visit at that time to the University of Washington by Dr. W. A. Murrill; the other, an incident which occurred one day as Dr. and Mrs. Zeller were rowing on the Sound in search of botanical specimens. Landing in a small cove roofed by an overhanging rock in whose crevices the western maidenhair fern was abundant, they found embedded among the rhizomes numerous glistening clay-colored fruiting bodies of an unknown fungus which later proved to be an undescribed species of *Rhizopogon*. Subsequently it was published under the name *R. diplophloeus* Zeller and Dodge. This new species of a genus which is largely hypogaeous served as his introduction to the intriguing subterranean basidiomycetes upon which he became an undisputed authority. Naturally and necessarily his interest widened to cover the entire field of Gasteromycetes, of which a contemporary internationally known mycologist writes, "He had, in my estimation, the best first-hand knowledge of the group as a whole of any man in the United States or Canada. And he was a world-recognized authority." Another mycologist, likewise world-known, states, "In his chosen field, the Gasteromycetes, Dr. Zeller was first among living authorities. The better-known groups, like the puffballs and phalloids, have been admirably treated a number of times. But the field studies which were made possible by the riches of his own Northwest, and the painstaking investigation of odd or imperfectly understood forms from elsewhere, made his knowledge unique. In a day when most of us are happy to be able to claim aquaintance merely with one or another *-aceae* he was that rare and heartwarming phenomenon, a Good Mycologist."

In spite of his attainments in this field, the Gasteromycetes represented, of necessity, a side-issue professionally, for by appointment he was a plant pathologist and in constant touch with the need for pathological research in the Northwest. During his twenty-nine years in Oregon, he assisted in solving many knotty problems relating particularly to berry and tree-fruit production in the state.

A glance at the appended list of his publications will suffice to indicate somewhat the variety of problems involved and the value, to the fruit-growers and farmers, of his investigations. Strawberries and cane-fruits, pomaceous and drupaceous tree-fruits, all came in for their share of attention; and Northwestern orchards are cleaner and more profitable because of his work and that of his associates. But his interest in pathology was not confined to crop plants. Any leaf-spot or other indication of disease caught his eye, and required of him an investigation into its life history. Note, for instance, such items as: "New or noteworthy fungi on Ericaceous hosts in the Pacific Northwest"; "An anthracnose of *Ledum*"; "A witches' broom of Ocean Spray."

More than one hundred fifty scientific papers were prepared for publication by Dr. Zeller, alone or in collaboration with other authors, most of the subjects relating to fungi. Outstanding among these are his contributions to a knowledge of the Gasteromycetes. The following mathematical data are more than mere figures, for into them can be read something of Dr. Zeller's familiarity with the fields in which he worked, his persistent and consistent application to research in spite of difficulties, and his scientific curiosity which gave him no rest. Independently he described three new orders, nine families, six genera, eighty-one species; and established twenty-nine new names and combinations. Added to these are three new genera, sixty-two species, and fifty-nine combinations published in co-operation with other scientists.

During his last year, he spent a four months' sabbatical leave at the New York Botanical Garden, preparing the comprehensive treatment of the Gasteromycetes which had been requested of him for inclusion in the North American Flora. Fortunately he was privileged to complete considerable of the manuscript, particularly the very important keys whose publication has given to mycologists one of his conspicuously important contributions to science.

The hypogaei were perhaps his best love. Always in his car he carried a short-handled rake, and he was rarely known to pass a likely piece of woods without stopping to do a little tentative grubbing. He was one of those rare collectors of these elusive fungi who never came away empty-handed. The ability to discover subterranean fungi involves principally three characteristics

—an understanding of their requirements, good judgment in applying this understanding, and luck. The order and proportions of these three factors are not fixed, and many an unsuccessful grubber would like to believe that the third is paramount. Whatever the case, Dr. Zeller invariably brought home a handful or two of fruiting bodies—not only of his own group but also, frequently, of those hypogaeous ascomycetes, the Tuberales, generally much rarer.

Concerning his collecting of subterranean fungi, Dr. Zeller always had a new good story on himself. On one occasion he, with a party of other enthusiastic collectors ("groundhoggers" was the official name), thoroughly worked over a mixed grove of conifers and deciduous shrubbery. When they had finished, it must be admitted that with the moss rolled back and the duff in large quantities removed from beneath the trees, the woods looked considerably worse for wear and certainly must have been perplexing to any passing layman. Having occasion, in line with his duties as pathologist, to visit fruit-growers in the vicinity sometime later, he was startled to be informed of a recent cougar hunt organized by the farmers of the community. His surprised exclamation brought the reply, "Yes, sir, a few weeks ago we found the grove yonder all clawed up, and nothing smaller than a cougar could have done it. So we all went out for a day with our guns—but the critter's still at large." When Dr. Zeller recounted the story and his friends asked, superfluously, whether he had enlightened the farmers, his deep chuckle was sufficient answer to the foolish question.

Dr. Zeller was a member of the American Mycological Society, of which last fall he was elected vice-president; of the Phytopathological Society of whose Pacific Division he was president from 1922 to '24, and a member of its advisory board from 1925 to '28. He was associate editor of its official organ, *Phytopathology,* from 1924 to '30; was a member of Alpha Sigma Delta; and was elected to honorary membership in Phi Sigma. On the Oregon State College campus he served for a year as president of the local chapter of the Sigma Xi; a year as president of the Phi Kappa Phi chapter, and was its delegate to the national convention in 1933. In 1913 he was a member of the Alaska kelp expedition conducted by the Bureau of Soils, U.S.D.A.

Though his professional responsibilities and accomplishments would appear to require a full-time schedule, he somehow found time for other important activities. A member of the official board of the local Methodist church, he helped carry through the construction of the impressive present building of that denomination; and for twenty-two years he was chairman of the advisory committee of Wesley Foundation, which has done outstanding service for college young people. Special honors were to have been accorded him at the twenty-fifth anniversary of this student group, but the date fell upon the day following his death.

With four daughters in the family, Dr. and Mrs. Zeller for years were actively interested in Camp Fire; and were so largely responsible for the effective organization and smoothly-running machinery of the Corvallis Council that in 1948 they were each granted the Luther Gulick award given volunteer workers for extraordinary service.

Properly to appraise Dr. Zeller's contributions to science and to the community, one must know that stalking all his activities was the illness which had followed him from childhood, had weakened his otherwise powerfully-built frame, and from which he never was completely free. He treated it casually; if he ever complained, no one knew it. "Patient through suffering" is the thought which now comes to his friends far more frequently than when he seemed naturally to hold his place among individuals of normal health. The sympathetic understanding and co-operation in his home and the companionship of his friends were of fundamental value to him. He will be missed as a scientist. Even more he will be missed by those who knew the twinkle of his eye, his ever-ready story, his deep infectious laugh, and the kindness of his heart.

OREGON STATE COLLEGE,
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LIST OF PUBLICATIONS BY S. M. ZELLER OR IN JOINT AUTHORSHIP

1. The development of *Stropharia ambigua*. Mycologia **6**: 139–145. *Illus.* 1914.
2. The development of the carpophores of *Ceriomyces Zelleri*. *Ibid*, **6**: 235–239. *Illus.* 1914.

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6. Studies in the physiology of the fungi. II. *Lenzites saepiaria* Fries, with special reference to enzyme activity. Ann. Mo. Bot. Gard. **3**: 439–512. *Illus.* 1916.

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8. (With C. W. Dodge) Rhizopogon in North America. Ann. Mo. Bot. Gard. **5**: 1–36. 1918.

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11. Fungi found on *Codium mucronatum*. Puget Sound Biol. Sta. Publ. **2**: 121–125. *Illus.* 1918.

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A NEMATODE–CAPTURING FUNGUS WITH ANASTOMOSING CLAMP–BEARING HYPHAE

CHARLES DRECHSLER [1]

(WITH 4 FIGURES)

Under the genus *Nematoctonus* I have described earlier five nematode-destroying fungi whose hyphae are liberally beset with clamp-connections of the kind long known as being characteristic of some groups in the Basidiomycetes. Four of these fungi, namely *N. tylosporus* (1), *N. leiosporus* (1), *N. pachysporus* (3), and *N. leptosporus* (3), attack in a rather commonplace parasitic manner by sending a germ tube into the animal host from an externally adhering conidium. Necessarily the conidium in these parasites, and, indeed, in the generality of fungous parasites subsisting on free-living terricolous nematodes, must adhere very firmly, for otherwise it would almost certainly be dislodged as the animal continues to move briskly through materials of close texture during the period required for penetration of the integument and slow transfer of spore contents into the invading hyphal tip. In *N. tylosporus* and *N. leptosporus* the conidium secretes a minute mass of glutinous material at its apex while it is still supported on its sterigma. However in these two species, as also in *N. leiosporus* and *N. pachysporus*, the adhesive organ most usually found operating effectively is formed after the spore has become detached, the fallen conidium then regularly putting forth a short, erect or ascending outgrowth with a glandular tip, or terminal glandular cell, that secretes a sizable globule of adhesive material. In *N. haptocladus* (4), the fifth member of the genus to be named and described, similar adhesive outgrowths or adhesive organs are formed

not only on fallen conidia, but also on the vegetative mycelium, usually being produced distally on prostrate hyphal branches as upcurving terminations. Rather often, especially when the proximal filamentous connection has been weakened through withdrawal of protoplasmic contents, the specialized hyphal termination is torn loose by a vigorous adhering eelworm, and then is carried away by the doomed animal much like a fallen conidium with glutinous outgrowth. Yet often, again, the hyphal connection withstands the struggles of the adhering eelworm, which consequently is held captive while undergoing invasion and, after death, expropriation of its fleshy substance. In view of the predacious manner of attack thus revealed, *N. haptocladus* offers an engaging parallelism with the few nematode-capturing phycomycetous forms found among the Zoopagaceae, as well as with the considerably more numerous nematode-capturing species that have been made known in the series of clampless predacious hyphomycetes.

Another species of *Nematoctonus* that attacks free-living terricolous eelworms both parasitically and predatorily came to light more recently in several maizemeal-agar cultures, which, after being overgrown with uncontaminated mycelium of *Pythium irregulare* Buism., had been further planted with pinches of decaying chess (*Bromus secalinus* L.) detritus taken from a handful of this material kindly gathered by Dr. W. J. Zaumeyer near Hermiston, Oregon, on August 20, 1947. The new *Nematoctonus* usually made its appearance ten to fifteen days after the addition of the decaying residues and in all instances obviously subsisted altogether on the many eelworms that soon had infested the cultures. Nearly all of the eelworms present belonged either to a somewhat robust species kindly identified by Dr. G. Steiner as *Panagrolaimus subelongatus* Cobb, or to a much more slender species determined as *Ditylenchus* sp. The two types of nematodes were utilized by the fungus without evident preference, though owing to its much greater abundance *P. subelongatus* was destroyed in larger numbers.

Unlike *Nematoctonus haptocladus,* which often is first observed developing in agar plate cultures at some distance from the decaying materials whence it originates, the Oregon fungus regularly begins its visible development in tracts bordering the planted detritus, and then spreads progressively into more remote areas.
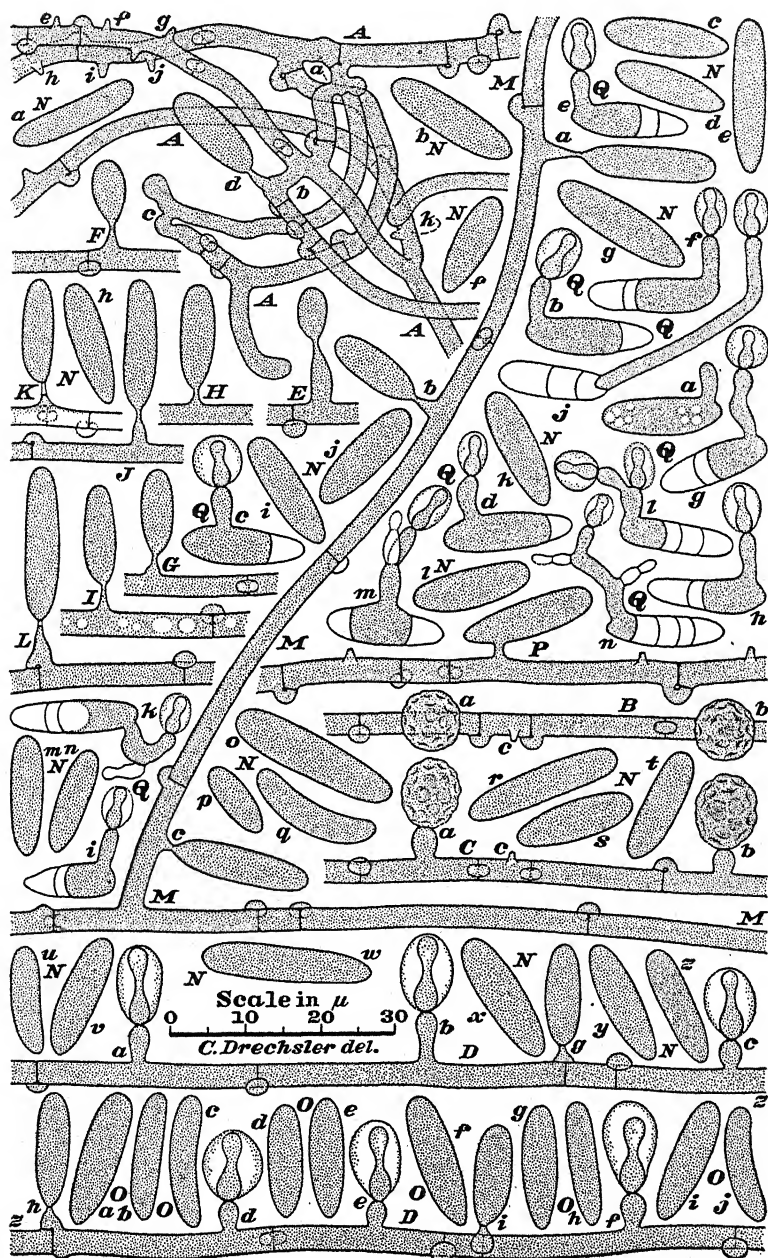
FIG. 1. *Nematoctonus concurrens.*

Nevertheless its ramifying mycelium (FIG. 1, *A*) greatly resembles that of *N. haptocladus* in general aspect, the constituent hyphae (FIG. 2, *A, a, b; B, a, b.* FIG. 3, *A; J, a, b; K, a–c.* FIG. 4, *A; B, a–c*) being filled while in their youthful condition with protoplasm of nearly homogeneous consistency.   The longer filaments are little given to abrupt changes either in width or in direction.   All mycelial elements except the shortest branches are studded with clamp-connections.   For the most part the clamps are spaced at distances of 20 to 80 μ, but here and there they are wont to occur in more crowded arrangement with some intervals not exceeding 2 μ.   Apparently no cross-walls other than those associated with clamp-connections are formed to serve as ordinary partitions between adjacent living undifferentiated vegetative hyphal segments.   Hyphal fusions (FIG. 1, *A, a–c.* FIG. 2, *A, c, d; B, c, d.* FIG. 3, *A, a; I, a; J, c; K, d, e.* FIG. 4, *A, a–c; B, d*), which have not been observed either in *N. haptocladus* or in any of the four congeneric parasites, occur here in sufficient numbers to be immediately noticed, though scarcely with the frequency of anastomoses in the clampless nematode-capturing hyphomycetes.

The mycelium of the Oregon fungus, like that of *Nematoctonus haptocladus,* is furnished with predacious organs consisting individually of a globose adhesive body borne aloft 2 to 7 μ above the substratum on a short stalk rising vertically from a prostrate hypha. When examined microscopically from above, in their undisturbed posture and without any addition of water, they appear as projecting knobs with a pitted or irregularly indented surface (FIG. 1, *B, a, b;* FIG. 2, *A, e–o;* FIG. 4, *A, d*) ; their internal make-up and underlying hyphal connections being then only indistinctly visible or sometimes even wholly obscured.   Viewed from the side in a dry mount the hyphal connections stand out clearly, but the internal make-up of the adhesive body still remains concealed (FIG. 1, *C, a, b*).   The composition of the predacious organ is seen better in material that has been slightly moistened and with gentle pressure covered with a cover glass; the adhesive body in material thus mounted presenting a smoothly rounded profile, and revealing within it an elongated cell conspicuously narrowed in the equatorial region somewhat after the manner of an hour-glass (FIG. 1, *D, a–f.* FIG. 2, *B, e–o; C, a, b; D, a, b; E, a–c; F, a–c; G, a–f; H, a; I, a–c; J, a;*

K, a; L, a–c; M, a.   fig. 3, *A, b; B, a, b; C, a, b; D, a, b; E, a;*
F, a; G, a, b; H, a, b; I, b, c; J, d–f; K, f–h.   fig. 4, *B, e–h; C, a*).
A very distinct line of demarcation always separates the distally
rounded protuberant stalk from the proximally rounded elongated
cell.   Although an abrupt junction of two rounded lobes might in
itself be expected to offer much the appearance of a septum even
where no septum exists, close examination of many predacious or-
gans has inclined me strongly to the belief that a delimiting cross-
wall is really present here—this cross-wall, of course, being an or-
dinary one, not associated with a clamp-connection.

Near its attachment to the stalk for a distance upward of about
1.5 μ, the peripheral wall of the elongated cell seems slightly thick-
ened and has a somewhat dark indurated appearance.   Beyond this
more substantial collar-like part the envelope is uniformly thin,
though yet of sufficient thickness to remain clearly visible through-
out; the fungus thereby differing from *Nematoctonus haptocladus,*
in which the membrane of the corresponding cell is so thin at the
rounded distal end as to be virtually indiscernible.   It may be pre-
sumed that whereas in *Nematoctonus haptocladus* the glutinous
substance surrounding the cell is probably given out mainly from
the very thin-walled apical region, exudation in the Oregon fungus
may take place about equally from all portions of the membranous
envelope above the indurated collar-like part.   The glandular cell
of the Oregon fungus is fully twice as long as that of *N. haptocladus,*
and, in general, about half again as wide.   Its narrower shape is
accentuated in some degree by its more gradual and more extended
median constriction.   Its greater volume and dimensions are re-
flected in correspondingly greater volume and dimensions of the
globule of adhesive material secreted by it; and its more elongate
form seems associated with a noticeably greater tendency toward
an elongated shape in the globule.

Some protuberant stalks after producing a glandular cell in the
usual way will grow out laterally or obliquely at the tip and give rise
on a short prolongation to a second glandular cell (fig. 3, *J, g*).
The process may be repeated again with the resulting development
of a third glandular cell (fig. 2, *H, b*).   Such successive develop-
ment, like the similar development noted earlier in *Nematoctonus
haptocladus* as well as in *N. pachysporus,* is frequently preceded by

Scale—μ
0 10 20 30
C.Drechsler del.
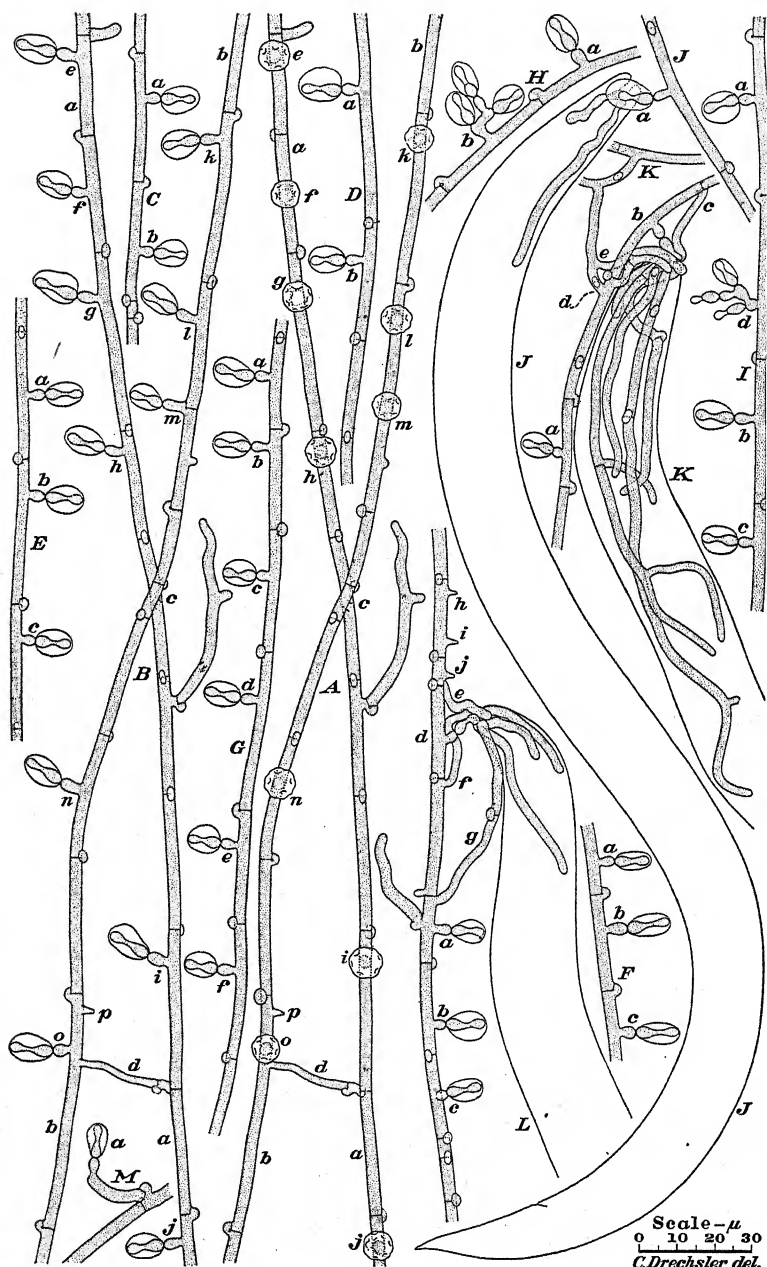
FIG. 2. *Nematoctonus concurrens.*

degeneration in the older glandular cells—this degeneration becoming manifest usually in withdrawal of protoplasmic contents from the distal lobe (FIG. 3, *J, h*), together sometimes with disappearance of the enveloping mass of adhesive material (FIG. 2, *I, d*). In instances where the distal lobe is found empty while the proximal lobe is still filled with protoplasm, the retaining wall present in the constriction consists of an ordinary partition not associated with a clamp-connection.

As the very short erect stalks supporting the glandular cells always arise from prostrate hyphae, never being borne on submerged or ascending mycelial filaments, the Oregon fungus, much like *Nematoctonus pachysporus,* is capable of capturing only nematodes moving on the surface of a culture.   In its usually forward movement, the animal commonly strikes the raised adhesive globule with the anterior portion of its body, often, indeed, being found held directly by its head (FIG. 2, *J, a.*   FIG. 3, *I, b, c; J, i; K, i.*   FIG. 4, *B, i; D–G: a; H, a*) ; though attachment to adhesive organs also takes place farther backward (FIG. 4, *B, j, k*).   Adhesion to a single predacious organ suffices, as a rule, for the capture of robust specimens of either *Panagrolaimus subelongatus* or *Ditylenchus* sp. After the struggles of the captive have diminished in violence, presumably from exhaustion, the glandular cell puts forth a narrow process which perforates the animal's integument, then immediately widens out and begins growing through the fleshy interior as an assimilative hypha (FIG. 3, *H, c;* FIG. 4, *D, a*).   In many instances the assimilative hypha soon gives off a branch close to its origin (FIG. 2, *J, a;* FIG. 4, *H, a*), but often, again, it may attain a length of approximately 100 μ while remaining in a simple condition (FIG. 3, *I, b, c; J, i.*   FIG. 4, *E–G: a*).   Branching in assimilative hyphae frequently is accompanied, or perhaps rather closely followed, by the formation of clamp-connections (FIG. 2, *K, b;* FIG. 3, *K, i*).

Although a single predacious organ usually is altogether effective in holding fast a moderately robust eelworm, the struggling animal often brushes against other predacious organs, and thus becomes affixed in two (FIG. 3, *H, b, c; I, b, c*), three (FIG. 4, *B, i–k*), or more places.   Since here, as in nematode-capturing fungi generally, each predacious organ operates rather independently of others, captives often incur multiple invasion by reason only of their multiple

affixture (FIG. 3, *I, b, c*). In the Oregon fungus multiple infection further comes about very frequently through the curious development of accessory infective branches (FIG. 2, *K, c–e; L, e–g.* FIG. 3, *I, d; J, j–l; K, j–m.* FIG. 4, *B, l, m; C, c; D–G: b, c; H, c*). These branches arise usually at distances of 10 to 50 μ from a predacious organ that has newly captured a nematode. Very often they are put forth from the same hypha that bears the predacious organ (FIG. 2, *K, c, d; L, e–g.* FIG. 3, *J, l.* FIG. 4, *B, l; C, c; D, b, c*), but with about equal frequency they originate from neighboring hyphae, which in some instances have an observable connection with the predacious organ either from the ramification of the mycelium (FIG. 2, *K, e*) or from anastomosis of mycelial filaments (FIG. 3, *K, j–m*), but in other instances lack such connection (FIG. 3, *J, j, k;* FIG. 4, *B, m*). Often the growth of the accessory branches is from the beginning rather accurately directed toward the position where the struggling animal is held fast by the fungus (FIG. 3, *J, l;* FIG. 4, *D, b*), though often, again, their courses seem rather haphazard or even circuitous (FIG. 4, *B, j; D, c*). Despite some aberration they converge unmistakably as they elongate (FIG. 4, *E, b, c*), and after about an hour or perhaps two hours of growth they reach their goal, each bringing its tip against the animal's integument in immediate proximity to the adhering predacious organ (FIG. 4, *B, l, m; F, b, c*). On penetrating the integument the individual branch intrudes a prolongation which then extends itself autonomously through the fleshy interior as an assimilative hypha (FIG. 4, *C, c; F, b; G, b*) in fellowship with the assimilative hypha arising from the predacious organ.

Although in most cases the number of accessory infective branches associated with a predacious organ ranges from one to five, development of such branches in numbers from six to ten occurs rather often, and instances of development in numbers from eleven to fifteen come under observation at least occasionally. The convergence of multiple branches makes for an appearance strongly reminiscent of sexual reproductive apparatus in species of oomycetes where the oogonium is fertilized by plural antheridia. No development of accessory infective branches was recorded in my account of *Nematoctonus haptocladus* or in the descriptions of the four congeneric species, though until further observations are made

the possibility is not to be dismissed that among these closely related forms similar development might occur on so small a scale as readily to escape detection. The accessory infective branches produced by the Oregon fungus offer some little resemblance to the supplementary connections formed in some clampless nematode-capturing hyphomycete species—my *Dactylella doedycoides* and my *D. heterospora* (2: 341–342) may be cited as examples—where the stalk supporting the predacious organ lacks sturdiness, owing mainly to its length, and consequently often suffers injury from the struggles of the prey. These supplementary connections, however, are commonly produced only singly and, as a rule, are intercalated between two fungus cells external to the animal. Unlike the accessory infective branches, they generally neither penetrate the animal's integument anew nor intrude an additional assimilative hyphal system; their function evidently being only to supply better communication between the external mycelium and the assimilative hyphae intruded by the predacious organ.

Since in most instances a captured specimen of *Panagrolaimus subelongatus* adheres to the predacious organ by its head, invasion of its body proceeds commonly from the head (FIG. 2, *J–L;* FIG. 3, *I–K;* FIG. 4, *D–G, H*) toward the tail. The angular relationships of the ramified mycelium in the posterior portion of a dead captive (FIG. 3, *L;* FIG. 4, *I, J*) suggest strongly that hyphal fusions must occur rather frequently among assimilative filaments, even though it is true that an appearance of hyphal fusion is often simulated here through the circumstance that many branches are extended in a direction opposite to the direction of growth of the parent filament. As a rule the protoplasm elaborated by the fungus from the contents of a relatively small eelworm is transferred to the external mycelium entirely through the adhesive organ and accessory infective branches. This is often true also where larger animals are concerned, if many accessory infective branches are present. However, in many instances where the eelworm is of large size and only a few accessory infective branches are present, a portion of the elaborated protoplasm is used in extending several hyphae—often about five or six—out from the posterior (FIG. 3, *L, a, b;* FIG. 4, *J*) and median portions of the animal. Obviously these erumpent hyphae belong with the

Fig. 3. *Nematoctonus concurrens.*

external mycelium, and therefore, after some elongation and branching, may put forth predacious organs and conidia.

In contrast to *Nematoctonus haptocladus,* which produces conidia only rather sparingly, the Oregon fungus usually shows abundant sporulation. Its prostrate mycelium gives rise to conidia haphazardly (FIG. 1, *A, d–k*), the longer procumbent hyphae often bearing them (FIG. 1, *D, g–i;* FIG. 2, *A, p; L, h–j*) interspersed among predacious organs. They originate as terminal swellings on tapering sterigmata (FIG. 1, *E, F*) and increase in size with continued accession of protoplasm from below (FIG. 1, *A, d; G–J*) until they become delimited from the slender supporting tip by a septum (FIG. 1, *K, L;* FIG. 4, *K*). On the ascending hyphae, which regularly are devoid of predacious organs, conidia are produced in the beginning at rather wide intervals (FIG. 1, *M, a–c;* FIG. 3, *M, a, b*), but seemingly more conidia are later put forth from intercalary positions, to provide eventually a bristling display (FIG. 4, *L, M*). Prostrate (FIG. 3, *K, b, c*) as well as aerial (FIG. 4, *N–P*) hyphae on which conidia have been produced at close intervals show in their denuded condition a conspicuously close arrangement also of clamp-connections.

Although slightly plumper and appreciably larger, the mature detached conidia of the Oregon fungus (FIG. 1, *N, a–z; O, a–j.* FIG. 3, *N–P: a–z.* FIG. 4, *Q, a–z*) most nearly resemble, among congeneric forms, those of *Nematoctonus haptocladus* with respect to size and shape. In my cultures they germinated less freely than the conidia of *N. haptocladus,* but their germinative development followed essentially the same course. The fallen spore would first send up a short erect outgrowth from a position usually near one of its ends, as simultaneously several clustered vacuoles came into view toward its other end (FIG. 1, *Q, a*). On the rounded tip of the spur-like outgrowth a glandular cell, noticeably smaller but otherwise similar to glandular cells of mycelial origin, was then produced while the farther end of the spore was emptied of protoplasmic contents with concomitant formation of one (FIG. 1, *Q, b–d*) or two (FIG. 1, *Q, e–i*) retaining walls. In one observed instance (FIG. 1, *Q, j*) the spore envelope was almost wholly emptied from production of a single glandular cell at the tip of a germinative outgrowth over 30 μ long—this being fully six times the usual length

of the erect support. Ordinarily, however, about one-half to three-fifths of the protoplasm was left over, so that the conidium through further withdrawal of contents (FIG. 1, Q, k) and deposition frequently of a third retaining wall (FIG. 1, Q, l) was capable of giving rise to a second glandular cell on a short prolongation of the germinative outgrowth. Occasionally a conidium that had produced a second glandular cell on an outgrowth arising from a median position showed an empty segment at each end (FIG. 1, Q, m). Following the degeneration of the first two glandular cells produced by them some conidia extended their outgrowth again to form a third glandular cell (FIG. 1, Q, n), thereby emptying a fourth segment though yet retaining about one-fifth of their original protoplasmic contents—enough, presumably, to have made possible the formation later of a fourth glandular cell.

Each glandular cell produced on a germinative outgrowth soon secretes an enveloping globule of adhesive material which serves, whenever opportunity offers, to attach the conidium to some roving eelworm (FIG. 4, H, b). An animal that has been thus encumbered may continue to move about until it is disabled from progressive invasion by an assimilative hypha intruded by the adhering spore, but rather often before such disablement supervenes it encounters a predacious organ and is held fast (FIG. 4, H, a). In the latter event the glandular cell produced by the conidium may evoke development of accessory infective branches (FIG. 4, H, c) after the same manner as glandular cells of mycelial origin. As the very numerous conidia in my cultures gave rise to adhesive outgrowths only sparingly, the Oregon fungus displayed little of the strong capabilities for parasitic attack it assuredly should unfold under conditions favorable for germination of its spores. Owing to the enduringly sturdy attachment of its predacious organs, including those borne on lateral branches (FIG. 2, M, a; FIG. 3, F, a; G, a, b), it affords little scope for the simulation of parasitic attack usual in *Nematoctonus haptocladus*, where the attachment of predacious organs often becomes weakened early through evacuation of the proximal segments of the branches on which they are borne.

Occasionally a fallen conidium is found united to a neighboring mycelial filament by means of a short hyphal connection (FIG. 1, P). Manifestly such a fusion is of the same character as the hyphal
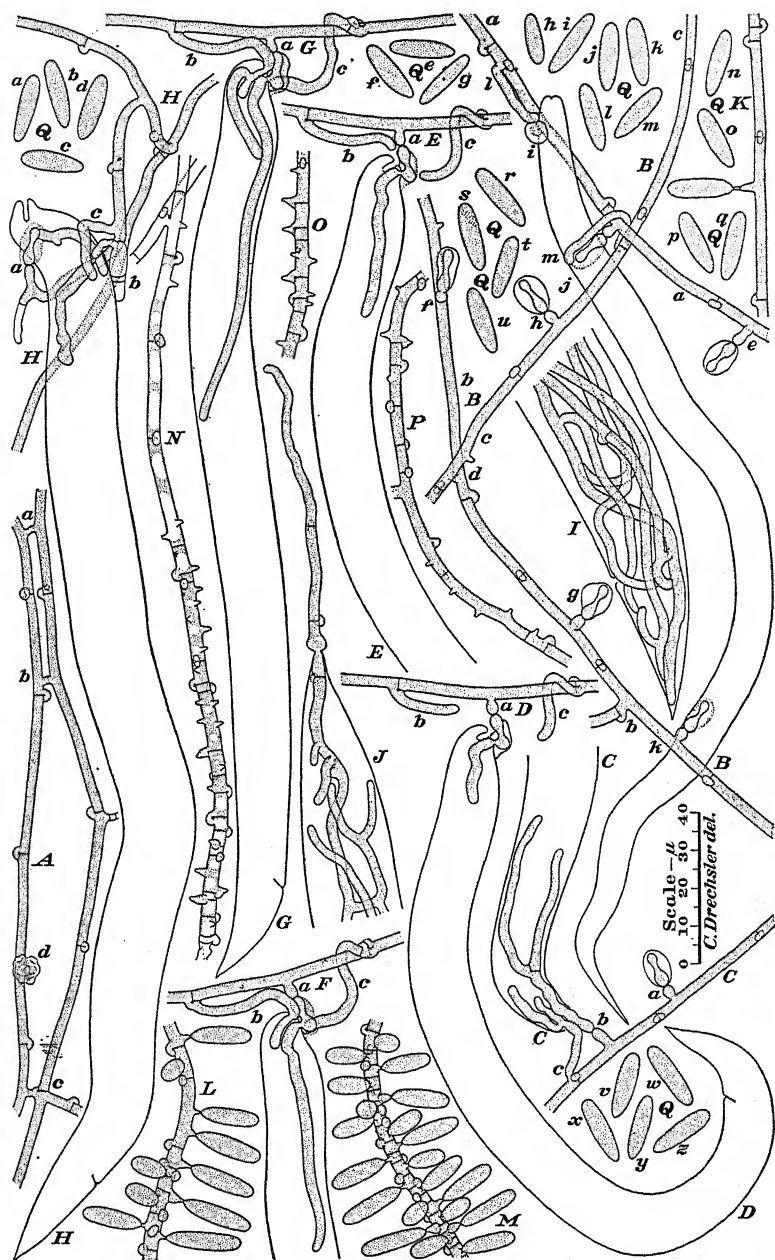
FIG. 4. *Nematoctonus concurrens.*

anastomoses so frequent in the fungus, and like these offers parallelism with the clampless nematode-capturing hyphomycetes. Since hyphal fusions serve to join numerous small mycelia into more extensive three-dimensional tracts, and in some basidiomycetes bring about nuclear relationships necessary for sexual development, the Oregon fungus would seem, perhaps somewhat more likely than the several congeneric forms previously described, to yield a basidial stage visible to the naked eye. In my cultures, however, no basidial stage ever came to light, nor was any reproductive apparatus ever found produced by the fungus apart from the conidial apparatus herein discussed. The fungus is therefore described in the genus *Nematoctonus,* and under a specific epithet having reference to the convergence of its accessory infective branches.

Nematoctonus concurrens sp. nov.

Mycelium tenellum, oculo nudo parum visibile, aliquid araneosum. Hyphae procumbentes incoloratae, filiformes, ramosae, in modum Hymenomycetum septato-nodosae, saepe inter se conjunctae, 2–3.5 μ crassae, cellulas glutinosas in apice columellarum atque conidia in apice sterigmatum gignentes; his columellis erectis, plerumque 3–7 μ altis, basi circa 2 μ crassis, sursum 2.4–3.7 μ crassis, apice rotundis; cellulis glutinosis harum 6–12 μ (saepius circa 10 μ) longis, 2.5–4 μ crassis, medio gradatim sed valde constrictis itaque ibi modo 0.8–1.5 μ crassis, primo nudis sed mox pila glutinis globosa vel ellipsoidea 6–12.5 μ longa, 4–8.5 μ crassa circumdatis, denique saepe ad vermiculum nematoideum inhaerentibus, itaque animal capientibus, cuticulam ejus perforantibus, quandoque tantummodo ipsis quandoque cum 1–15 ramulis adjunctis 10–50 μ longis hyphas assumentes incoloratas ramosas septato-nodosas intrudentibus quae carnem exhauriunt et interdum ramos mycelii extra emittunt. Hyphae ascendentes incoloratae, parce ramosae, septato-nodosae, 2–3.5 μ crassae, conidia in apice sterigmatum ferentes; sterigmatibus interdum ex nodis oriundis, 1.8–7 μ longis, basi 1.2–2.5 μ latis, sursum attenuatis, apice 0.6–0.8 μ latis. Conidia incolorata, cylindracea vel elongato-ellipsoidea, recta vel leniter curvata, sursum late rotundata deorsum similia vel leviter attenuata, plerumque 10–23 μ longa, 3.6–5.6 μ crassa, primo continua et protoplasmatis omnino repleta, post disjunctione ex apice columellae germinationis cellulam glutinosam ferentia, deinde quandoque identidem prope apicem columellae recrescentia 1 vel 2 alias cellulas glutinosas gignentia, denique in magna parte vel omnino inania et 1–4 septis intus divisa; cellulis glutinosis germinationis 4.5–6.5 μ longis, 2–2.5 μ crassis, medio constrictis, pila glutinis globosa vel ellipsoidea 4–7 μ longa 3.5–6.5 μ crassa mox vestitis.

Panagrolaimum subelongatum et *Ditylenchum* sp. capiens consumensque habitat in foliis caulibusque Bromi secalini putrescentibus prope Hermiston, Oregon.

Mycelium delicate, arachnoid, faintly visible to the naked eye. Prostrate hyphae colorless, filamentous, branched, 2 to 3.5 μ wide, studded with clamp-connections and often joined to one another by anastomosing connections, at variable intervals giving rise on protuberant outgrowths to glandular cells and producing conidia on the tips of sterigmata; the protuberant outgrowths regularly erect, mostly 3 to 7 μ high, about 2 μ wide at the base and 2.4 to 3.7 μ wide toward the rounded tip; the glandular cells here 6 to 12 μ (usually about 10 μ) long, 2.5 to 4 μ wide, gradually but strongly constricted at the middle and thus measuring only 0.8 to 1.5 μ in that region, at first naked but later always surrounded by a globose or ellipsoidal mass of glutinous material 6 to 12.5 μ long and 4 to 8.5 μ wide, therewith often adhering to a passing nematode, thus capturing the animal, and then, after perforating its cuticle, giving rise internally, sometimes alone and sometimes in association with 1 to 15 convergent accessory infective branches usually 10 to 50 μ long, to colorless, branched, clamp-bearing assimilative hyphae 2 to 3.5 μ wide, which permeate the fleshy body lengthwise, appropriating its substance, and in some instances eventually pushing branches out through the cuticle to elongate externally. Ascending hyphae studded with clamp connections, colorless, sparingly branched, 2 to 3.5 μ wide, bearing conidia on conical sterigmata usually 1.8 to 7 μ long, 1.2 to 2.5 μ wide at the base, and 0.6 to 0.8 μ wide at the tip. Conidia colorless, cylindrical or elongated ellipsoidal, straight or slightly curved, broadly rounded at the distal end, often rather similarly rounded at the basal end though sometimes slightly narrowed proximally, mostly 10 to 23 μ long and 3.6 to 5.6 μ wide, at first continuous and filled throughout with protoplasm, after abjunction producing a glandular cell on the tip of a germinative protuberant outgrowth, then frequently, following successive elongation of the outgrowth, giving rise to 1 or 2 additional glandular cells, thereby becoming largely or wholly emptied of protoplasm and concomitantly partitioned by 1 to 4 cross-walls; the glandular cells of germinative origin measuring usually 4.5 to 6.5 μ in length and 2 to 2.5 μ in width, soon becoming surrounded by a globose or elongated ellipsoidal mass of adhesive material commonly 4 to 7 μ long and 3.5 to 6.5 μ wide.

Capturing and consuming *Panagrolaimus subelongatus* and *Ditylenchus* sp., and besides often parasitizing them, it occurs in decaying leaves and stems of *Bromus secalinus* near Hermiston, Oregon.

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EXPLANATION OF FIGURES

Fig. 1. *Nematoctonus concurrens,* drawn to a uniform magnification with the aid of a camera lucida; × 1,000 throughout. *A,* Portion of clamp-bearing mycelium from surface of agar plate culture: *a–c,* anastomoses of hyphae; *d,* sterigma bearing a conidium with which it is still continuous; *e–k,* sterigmata from which the conidia have become detached. *B,* Portion of prostrate hypha as seen undisturbed in a dry preparation when viewed directly from above: *a, b,* globules of adhesive material showing irregular pitting of surface whereby the glandular cell and supporting stalk are concealed from view; *c,* denuded sterigma. *C,* Same portion of clamp-bearing prostrate hypha as seen in a dry preparation when viewed from side: *a, b,* globules of adhesive material whose pitted surface obscures the glandular cell within, each thereby offering a false appearance of having been formed terminally by the supporting stalk; *c,* denuded sterigma. *D,* Portion of clamp-bearing prostrate hypha (for lack of space drawn in two parts connecting at point *z*) as seen in a moist preparation under a cover glass: *a–f,* erect predacious organs, showing the erect spurs supporting aloft the hour-glass-shaped glandular cell surrounded by the globose mass of adhesive secretion; *g–i,* sterigmata, each bearing a conidium. *E, F,* Portions of clamp-bearing prostrate hypha, each with an erect sterigma terminating in a young growing conidium. *G–J,* Portions of clamp-bearing prostrate hypha (though no clamp is shown in *H*), each with an erect sterigma still continuous with the full grown or nearly full grown conidium surmounting it. *K, L,* Portions of clamp-bearing prostrate hypha, each with an erect sterigma on whose empty tip is borne a mature conidium. *M,* Portion of clamp-bearing prostrate hypha with the proximal portion of an ascending branch, which is likewise studded with clamp-connections and bears three conidia, *a–c,* all about fully grown though not yet delimited at the base. *N (a–z), O (a–j),* Detached mature conidia, showing usual variations in size and shape. *P,* Portions of prostrate hypha, beset with clamp-connections and denuded sterigmata, which has anastomosed with a detached conidium nearby. *Q,* Conidia showing various stages of germination: *a,* conidium with several small vacuoles and young germ tube; *b–d,* conidia, each with an empty segment at one end and bearing at the other end an erect spur that supports a glandular cell surrounded by a globule of adhesive material; *e–i,* conidia, each with two empty segments at one end and bearing near the other end an erect spur surmounted by a glandular cell which is surrounded by a globule of adhesive material; *j,*

conidium with envelope almost entirely emptied of protoplasm, and containing three partitions, its contents having been used in the production of an unusually long germ hypha whereon is supported an adhesive glandular cell; *k,* conidium with two empty segments at one end, and bearing at the other end a germ tube, which, after degeneration of the first adhesive glandular cell produced by it, continued growth to produce a second; *l,* conidium with three empty segments at one end, its remaining live segment being continuous with an erect germ tube whereon two glandular adhesive cells are supported; *m,* conidium with an empty segment at each end, its living median segment being continuous with a germ tube whereon two glandular adhesive cells have been formed, the older, proximal one having already degenerated; *n,* conidium with four empty segments, its remaining live segment being continuous with a germ tube that has formed three glandular adhesive cells successively, the older two, however, having now degenerated.

FIG. 2. *Nematoctonus concurrens,* drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A,* Portions of two main prostrate clamp-bearing hyphae, *a* and *b,* as seen from above, in a dry preparation not covered with a cover glass; *c,* short anastomosing connection joining the two main hyphae where they cross one another; *d,* longer anastomosing connection between the two main hyphae; *e–o,* globules of adhesive material with irregularly indented surface whereby is obscured the glandular cell within; *p,* denuded sterigma. *B,* Same two portions of mycelial hyphae *a* and *b* as seen in a moist preparation flattened down under a cover glass; the anastomosing connections *c* and *d* showing little change, but the predacious organs *e–o* here appearing in longitudinal profile; *p,* denuded sterigma. *C, D,* Portions of prostrate clamp-bearing hyphae as seen in a moist covered mount, each portion showing two predacious organs, *a* and *b,* in longitudinal profile. *E, F,* Portions of prostrate clamp-bearing hyphae as seen in a moist covered mount, each showing three predacious organs, *a–c,* in longitudinal profile. *G,* Portion of clamp-bearing prostrate hypha as seen in a moist covered mount; *a–f,* six predacious organs shown in longitudinal profile. *H,* Portion of clamp-bearing prostrate hypha as seen in a moist covered mount; showing in longitudinal profile two predacious organs, one, *a,* bearing a single adhesive cell, the other, *b,* bearing three adhesive cells. *I,* Portion of clamp-bearing prostrate hypha as seen in a moist covered mount; showing in longitudinal profile four predacious organs, of which three, *a–c,* have each a single glandular cell, while the other, *d,* bears two degenerate adhesive cells in addition to the one adhesive cell in a functional condition. *J,* Portion of clamp-bearing prostrate hypha with a predacious organ, *a,* that has intruded two growing assimilative hyphae into a captured specimen of *Panagrolaimus subelongatus. K,* Portion of clamp-bearing prostrate mycelium showing one predacious organ, *a,* in an inactive state, and another such organ, *b,* which after capture of a specimen of *P. subelongatus* has intruded an assimilative hypha into the animal; *c–e,* three convergent supplementary infective branches, each of which has intruded an assimilative prolongation into the captive. *L,* Portion of clamp-bearing prostrate hypha showing in longitudinal profile three inactive predacious organs, *a–c,* and a fourth predacious organ, *d,* which after capture of a specimen of *P. subelongatus* has intruded an assimilative hypha into the

animal; *e–g*, three convergent supplementary infective branches whereof two have each extended an assimilative prolongation into the captive; *h–j*, denuded sterigmata. *M*, Portion of prostrate hypha with a clamp-connection that has given off a short branch terminating in an erect predacious organ, *a*.

Fig. 3. *Nematoctonus concurrens*, drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A*, Portion of clamp-bearing prostrate mycelium as seen in a moist covered mount; *a*, anastomosis of two hyphae; *b*, predacious organ in longitudinal profile. *B*, *C*, Portions of clamp-bearing prostrate hyphae as seen in a moist covered mount, each showing two predacious organs, *a* and *b*, in longitudinal profile. *D*, Portion of clamp-bearing mycelium, showing two predacious organs, *a* and *b*, in longitudinal profile. *E*, *F*, Portions of clamp-bearing prostrate hyphae, each with a lateral branch terminating in an erect predacious organ, *a*. *G*, Long clamp-bearing hyphal branch as seen in a moist covered preparation; showing two predacious organs in longitudinal profile, one, *a*, being attached laterally, the other, *b*, being borne terminally. *H*, Portion of clamp-bearing prostrate hypha bearing a partly degenerated predacious organ, *a*, and two other predacious organs, *b* and *c*, that have been operative in capturing a small specimen of *Ditylenchus* sp.; from *c* a short assimilative hypha has begun invasion of the animal. *I*, Portion of prostrate clamp-bearing mycelium, showing two hyphae joined together by a short anastomosing connection, *a;* two predacious organs, *b* and *c*, after being operative in capturing a specimen of *Panagrolaimus subelongatus*, have each extended an assimilative hypha into the animal; two convergent supplementary infective branches, *d* and *e*, have begun growing toward the captive. *J*, Portion of clamp-bearing prostrate mycelium comprising a nearly simple hypha, *a*, and a ramified hyphal system, *b*, whereof two main branches are united by an anastomosing connection, *c;* of the five inactive predacious organs, *d–h*, shown arising from the mycelium in longitudinal profile, three (*d–f*) have a single glandular cell, one (*g*) has two adhesive cells, and the fifth (*h*) has three adhesive cells, the lowermost one having in part degenerated; the predacious organ *i*, having been operative in the capture of a specimen of *P. subelongatus*, has sent an assimilative hypha into the animal; of the three convergent supplementary infective branches present, *j–l*, one likewise has intruded an assimilative hypha. *K*, Portion of clamp-bearing prostrate mycelium comprising three hyphae, *a–c*, joined together by the anastomosing connections *d* and *e; f–h*, inactive predacious organs, each having a single glandular cell; *i*, predacious organ that has been operative in capturing a specimen of *P. subelongatus*, and has intruded a branching assimilative hypha into the animal; *j–m*, convergent supplementary infective branches that have reached the animal and, with the exception of *k*, have each intruded an assimilative prolongation into it; numerous denuded sterigmata are shown on hypha *b*, and a few on hypha *c*. *L*, Posterior portion of a dead captured specimen of *P. subelongatus*, showing presence of clamps on assimilative hyphae, and external prolongation of two branches *a* and *b* that have broken out through the animal's integument. *M*, Portion of clamp-bearing ascending hypha with two sterigmata bearing the conidia *a* and *b*. *N–P*, Detached mature conidia, *a–z*, showing usual variations in size and shape.

FIG. 4. *Nematoctonus concurrens,* drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A,* Two clamp-bearing prostrate hyphae joined together by three short anastomosing connections, *a–c; d,* a predacious organ as seen in a dry uncovered mount. *B,* Portion of prostrate mycelium including three clamp-bearing hyphae, *a–c,* two of which are joined together by a short anastomosing connection, *d;* besides four predacious organs in an inactive state, *e–h,* there are present three predacious organs, *i–k,* that have operated in the capture of a specimen of *Ditylenchus* sp.; the two active organs *i* and *j* having invited development of the two convergent supplementary infective branches *l* and *m,* respectively. *C,* Portion of clamp-bearing prostrate hypha with one inactive predacious organ, *a,* and with another predacious organ, *b,* which after effecting the capture of a specimen of *Panagrolaimus subelongatus* has extended a branched assimilative hypha into the animal; a supplementary infective branch, *c,* besides has extended an assimilative prolongation into the captive. *D,* Portion of prostrate clamp-bearing hypha with a predacious organ, *a,* that after having effected capture of a specimen of *P. subelongatus* has sent a short assimilative hypha into the animal; two convergent supplementary infective branches, *b* and *c,* have begun growing toward the captured eelworm. *E,* Same hypha and captured nematode twenty minutes later; showing continued growth of the intruded assimilative hypha, and also further growth of the two convergent branches toward the place where the animal is affixed to the fungus. *F,* Same hypha and captured nematode one hour later than in *E;* showing further growth of the assimilative hypha extended from the predacious organ, and the intrusion nearby of an assimilative hypha from the convergent branch *b;* the convergent branch *c* has elongated only a little. *G,* Same hypha and captured nematode one hour later than in *F;* showing additional growth of the assimilative hypha first intruded and both growth and branching of the assimilative hypha coming from convergent branch *b;* as the convergent branch *c* has not elongated further, it has not yet penetrated into the animal. *H,* Portion of clamp-bearing prostrate mycelium with a predacious organ, *a,* which after being operative in capture of a specimen of *P. subelongatus* has extended an assimilative hypha into the animal; another assimilative hypha was at the same time intruded nearby from a predacious organ borne on a conidium, *b,* which consequently is empty of protoplasm; a supplementary infective branch, *c,* meanwhile grew to the place where the predacious organ from the conidium had penetrated into the captive. *I,* Posterior portion of dead captured specimen of *P. subelongatus,* showing branching and anastomoses of assimilative hyphae. *J,* Posterior portion of a dead captured specimen of *P. subelongatus* showing branching of assimilative hyphae, and prolongation externally of a filament that broke out through the integument at the tip of the animal's tail. *K,* Portion of prostrate clamp-bearing hypha with a sterigma bearing a mature conidium. *L, M,* Portions of strongly ascending aerial hyphae, showing origin of conidia at close intervals and close arrangement of clamp-connections. *N–P,* Portions of ascending clamp-bearing hyphae showing denuded sterigmata and rather closely spaced clamp-connections. *Q,* Random assortment of detached conidia, *a–z,* showing usual variations in size and shape.

OBSERVATIONS ON STREPTOMYCES GRISEUS. IV. INDUCED MUTATION AND STRAIN SELECTION *

Eugene L. Dulaney, Myrle Ruger and Charles Hlavac

(with 2 figures)

One of the approaches to increasing the broth potency of a substance produced by a fermentation involves the putting into production of high yielding strains of the organism used in the fermentation. Such strains may be isolated from nature or may be natural or induced variants selected from strains already in use. The success possible with this approach is well exemplified by the published results of recent research on the development of *Penicillium notatum* and *P. chrysogenum* strains with an increased capacity for penicillin production (1, 7).

The use of strain selection and induced mutation would seem to offer possibilities for increasing yields of streptomycin. The variability of actinomycetes is well known and streptomycin producing cultures are no exceptions. Schatz and Waksman (8) have already called our attention to the variability of *Streptomyces griseus.* Carvajal (2), in addition, has isolated numerous strains of this organism from nature. The above authors mentioned streptomycin production as one of the characters in which variation was noted. Moreover, Stanley (9) has successfully used strain selection to increase yields of streptomycin. By use of ultraviolet light treatment of spores and single colony isolation, he was able to obtain strains which produced striking yields when compared to the parent culture.

The present paper is concerned with some aspects of natural and induced variability in *Streptomyces griseus,* particularly in relation to streptomycin production.

METHODS

The original culture of *Streptomyces griseus* used in this work was received from Waksman's laboratory. Stock cultures were prepared by adding spores to sterile soil. As cultures were needed, spores were transferred from these soil stocks to Blake bottle slants of yeast extract-glucose agar. After good sporulation was obtained, sterile distilled water was added to the bottle and loose spores were washed free. This spore suspension was decanted into a sterile flask, shaken vigorously for several minutes and then filtered through several layers of sterile absorbent cotton. The effect of ultraviolet light was determined by exposing a portion of this spore suspension to ultraviolet light at a wave length of 2,537 Å for varying intervals of time. As a rule the time interval employed was sufficient to allow killing of more than 99 per cent of the spores. Treatment with $(ClCH_2CH_2)_3N$ was achieved by mixing another portion of the spore suspension in phosphate buffer at pH 8.0 with the $(ClCH_2CH_2)_3N$ at a final concentration of 0.005 M. After the desired treatment, the spores were diluted in a solution of 1 per cent glycine in distilled water. Natural variation was investigated by plating out diluted suspensions of untreated spores. In all of the above instances, surviving spores were plated out on yeast extract-glucose agar. The colonies that developed were transferred to slants of the same medium and allowed to sporulate. Spores from these cultures were used to inoculate the fermentation medium. This medium consisted of soy bean meal 20.0 gr., dextrose 10.0 gr., sodium chloride 10.0 gr., and distilled water 1 liter. The pH was adjusted to between 7.0 and 7.2 with 1 N sodium hydroxide before sterilization. The medium was dispensed in 40 ml. amounts in 125 ml. Erlenmeyer flasks, plugged with non-absorbent cotton and autoclaved at 121° C. for 20 minutes. After cooling, the flasks were inoculated and placed on a rotary-type shaker moving at a rate of 220 rpm. so that each flask described a circle 1.5 inches in diameter. The flasks were incubated at 28° C.

The cylinder plate method, employing pure streptomycin hydrochloride as the standard, was used for assay throughout the investigations.
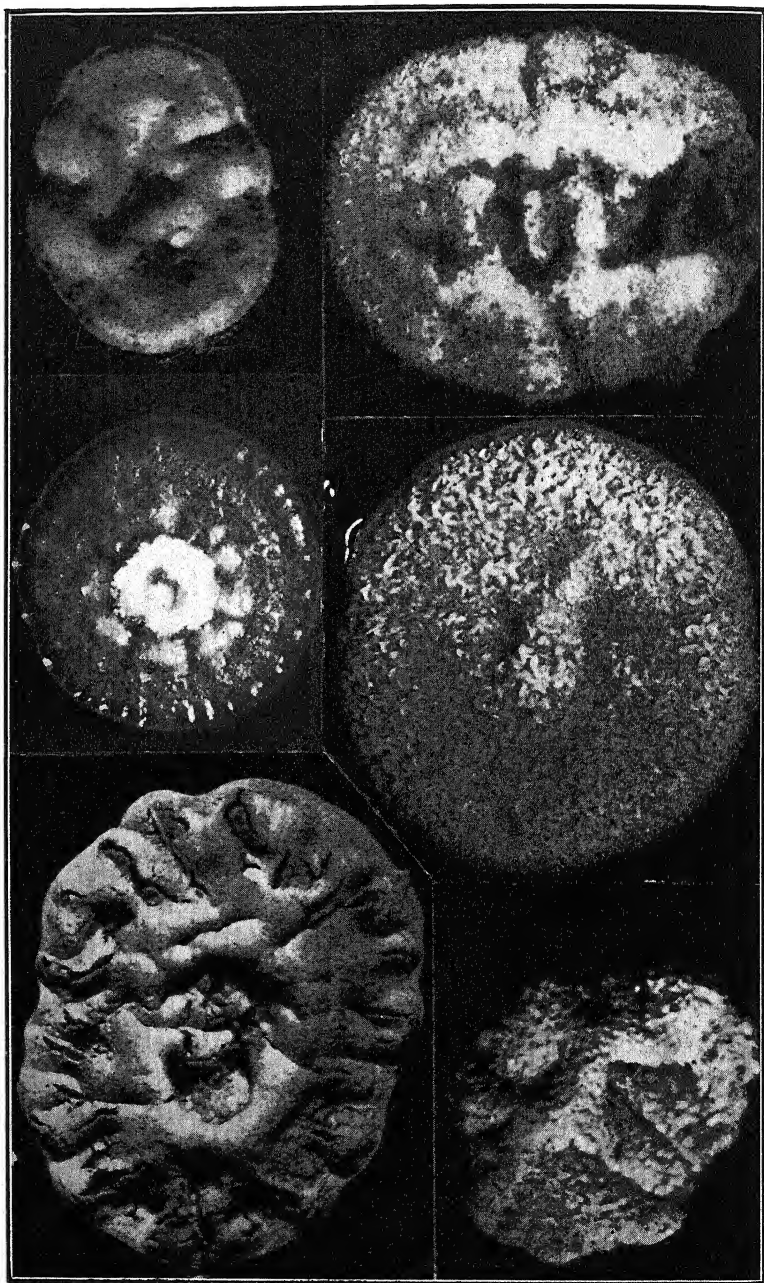
Fig. 1. Variants of *Streptomyces griseus;* all growing on yeast extract-glucose agar. ×4.

RESULTS AND DISCUSSION

Morphological variation. The amount of morphological variation depends upon the strain under observation. Some strains are morphologically quite stable, while others show a tremendous range of variation. Estimating the variation induced in these unstable strains as a result of treatment with ultraviolet light or $(ClCH_2CH_2)_3N$ is understandably difficult. It is quite obvious, however, that treatment of spores with both of these agents results in severe morphological change. Variants, that have not as yet been obtained from untreated spores, can be obtained after treatment. In addition, the incidence of the variants that are obtained without any treatment is increased by the above treatments. One of the most variable characters is the degree of sporulation. Cultures range from the parental type to types which form no spores at all. Not only the amount of sporulation but spore color is variable. The color range is from gray-green through light buff to pure white. This variation in amount of sporulation and in color of spores results in a marked variation in the colony appearance. Further changes can be seen in the margin and surface of the colony as well as in colony sectoring. The amount of exudate on the colony surface, as well as the amount and color of the soluble pigment released into the medium, varies among mutants. Changes in the amount and color of the pigment produced are often striking. The usual range in pigmentation is from none through a light yellow to a deep brown to black. Frequently, however, mutants are obtained which produce a diffusible pale pink to deep pink pigment. These observations were made with cultures growing on yeast extract-glucose agar. Media probably could be devised which would show these pigment changes to greater advantage.

Examples of ultraviolet light induced mutants are shown in figure 1.[1]

Attempts have been made to correlate productive capacity with morphological type. Detailed observations of a large number of mutants have shown some correlation but in no case is this correlation complete. One strain studied in detail was a near-albino

sporulating mutant which was quite stable morphologically. Study of more than 1,000 isolates from this culture resulted in one striking correlation between production and morphology. A number of cultures showed a ragged appearance with reduced sporulation and development of phage-like plaques. These cultures were significantly better than the parent, the correlation between production and morphological appearance being almost perfect.

Another strain studied in detail was a gray-green sporulating type. This was the most unstable culture that has thus far been examined. Two general types were isolated from it. One was the normal gray-green sporulating type which exhibited little variability in morphology or capacity for producing streptomycin. The other type could be conveniently termed albino, though it was certainly a heterogeneous mixture of albino types. Production by isolates of this latter group ranged from nothing to very high values. There was within this albino group no correlation between morphological type and streptomycin production. While it may be noted that all promising cultures isolated from this strain were of the albino type, it was likewise true that all isolates incapable of producing streptomycin were of the same type.

Results, then, would indicate that detailed investigation of one strain may reveal some correlation between productive capacity and morphological type. Such a correlation, however, will probably not hold true for a different strain. Thus far, no definite correlation between morphological character and productive capacity has been found. Although such a correlation may seem to exist if a relatively small number of isolates are investigated, it disappears if a detailed examination of large numbers of cultures is made.

Schatz and Waksman (8) have reported the isolation of cultures which produced no aerial mycelium and no streptomycin. From these cultures sporulating isolates were obtained which again produced streptomycin. Such cultures have been isolated in this and, undoubtedly, in other laboratories. Not all of these non-sporulating cultures, however, lack the ability to produce streptomycin. Some are capable of producing relatively high yields. In table I is shown the productivity of seven non-sporulating single colony isolates selected at random from a sporulating streptomycin producing strain.

Structurally these non-sporulating strains are probably composed of the substratum mycelium of Ørskov (6) or the primary mycelium of Klieneberger-Nobel (5). The lack of aerial or secondary mycelium may be due to the failure of the "initial cells," discussed by Klieneberger-Nobel (5), to organize.

Non-streptomycin producing strains. Sporulating strains, as well as non-sporulating strains, can be isolated which lack the ability to produce streptomycin. The incidence of these zero strains can be increased by treatment with ultraviolet light.

It was thought possible that the failure of these strains to produce streptomycin was due to their inability to synthesize various portions of the streptomycin molecule. If this were true it might be possible to obtain streptomycin production from these cultures by inoculating fermentation medium with spore mixtures of zero mutants which produced complementary portions of the streptomycin molecule. In addition, if the unsynthesized portion(s) of the streptomycin molecule was (were) supplied, single cultures might again produce streptomycin. These suppositions are predicated by the ability of the organism to unite the various portions of the molecule, when they are present, even though the portions under consideration might not be synthesized first and then united in the regular course of biosynthesis. However, when medium was inoculated with spore mixtures of these zero strains, no streptomycin was formed. In addition, when various parts of the streptomycin molecule were added singly and in combination to media inoculated with zero strains, negative results were again obtained.

It should be noted, however, that this work was done with only a few strains. The physiology of these strains would be similar if the formation of streptomycin were interrupted in each case by a mutation at the same gene position. In this event, similar intermediates would accumulate and the strains would react similarly to the addition of possible streptomycin precursors. In addition, if a fermentation medium is inoculated with a mixture of such cultures the negative results noted above are to be expected. Investigation of a group of cultures, in which the ability to synthesize streptomycin has been interrupted by mutations at different gene positions, may aid in isolating streptomycin intermediates and thus serve to determine the mechanism of biosynthesis.
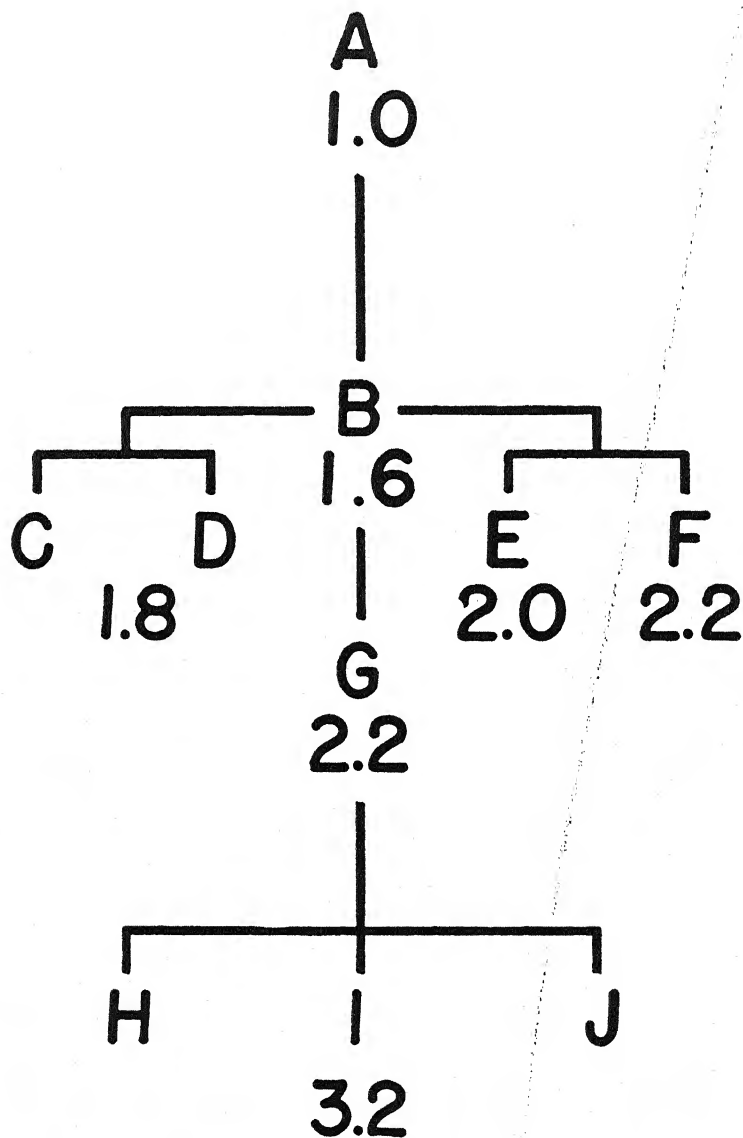
FIG. 2. Diagram illustrating increase in streptomycin production by successive isolation of mutants with increased capacity for production.

Strains with increased productive capacity. One of the reasons for undertaking a study of normal and induced variation in *Streptomyces griseus* was to determine if strains could be selected which possessed an increased capacity for streptomycin production. The results, in part, are summarized in figure 2. The broth potency produced by strain A is taken as unity and the productivity of selected strains is compared to strain A.

Spores from strain A were treated with ultraviolet light, and cultures which developed from the surviving spores were tested for streptomycin production. One of these cultures, B, produced 60 per cent more streptomycin than strain A, or 1.6 times as much. Spores from strain B were likewise treated with ultraviolet light. Two cultures thus obtained, C and D, produced approximately 12.5 per cent more streptomycin than the parent strain B, or 1.8 times as much as strain A. Although these yields are not striking, as compared with those obtained from the parent culture B, they represent a significant increase and are reproducible over a large number of retests. Spores of strain B were also treated with $(ClCH_2-CH_2)_3N$. Two cultures, E and F, which produced 25 per cent and 37.5 per cent more streptomycin than strain B, were so obtained. These strains produce 2.0 and 2.2 times as much streptomycin as strain A. These cultures, however, rapidly lost the ability to produce streptomycin, thus making further work with them impractical. The most promising culture isolated from strain B was strain G. This strain was obtained as a single colony isolate by plating untreated spores of strain B on yeast extract-glucose agar. It is morphologically similar to its parent and consistently produces streptomycin yields 37.5 per cent higher than the yields produced by the parent strain B, or broth potencies 2.2 times as high as are produced by strain A. Spores of strain G were treated with ultraviolet light, the survivors allowed to develop on yeast extract-glucose agar, and the resulting colonies isolated and tested. From approximately 150 isolates thus tested, three cultures were obtained which produced approximately 45.5 per cent more streptomycin than strain G. These cultures, H, I, and J, produce 3.2 times as much streptomycin as the original strain A.

Further mutation and selection of the highest producing strains has been carried out. The above results, however, are sufficient

to show how induced mutation and strain selection can be used to increase streptomycin production. When one is undertaking such selection, however, it must be understood that the cultures are being tested for production under the set of conditions being used. Cultures thus found to produce high yields should not necessarily be expected to produce corresponding yields with changed conditions. Changes in the fermentation environment may have varying effects on different strains. Medium specific mutants should be expected.

It would be very enlightening if the physiological changes resulting in increased streptomycin productivity could be determined. With this in mind, some investigations of the physiology of various mutants have been made. Although some physiological differences between various mutants have been found, nothing indicates why one strain is capable of producing more streptomycin than the other.

The improved broth potencies noted above are not confined to experiments made on media composed partially of complex plant and animal material. Relatively high yields have been obtained on well defined simple synthetic media (3) (4).

TABLE I

STREPTOMYCIN PRODUCTION BY NON-SPORULATING STRAINS OF
Streptomyces griseus

Culture Number	Maximum Yield γ/ml.	Day of Maximum
1	100	5
2	0	—
3	0	—
4	390	5
5	0	—
6	43	4
7	469	5

SUMMARY

1. The normal variation found in *Streptomyces griseus* can be increased by treatment with either ultraviolet light or $(ClCH_2-CH_2)_3N$.

2. Some of the variable characters include the color of spores and degree of sporulation; the surface and margin of the colony, as well as colony sectoring; the amount and color of exudate on the colony surface; and the amount and color of soluble pigment released into

the substratum. In addition, great variation occurs in the amount of streptomycin produced.

3. No complete correlation between morphological type and streptomycin production could be found.

4. Strain selection and induced mutation can be used to obtain strains with an increased capacity for producing streptomycin.

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THE EFFECT OF TEMPERATURE ON THE PAPILLATION OF OOGONIA OF ACHLYA COLORATA

HELEN SIMPSON REISCHER

(WITH 6 FIGURES)

Achlya colorata can be clearly distinguished from its closest relative, *Achlya racemosa,* by the difference in the diameters of their oospores as well as by differences in such minor features as relative abundance of gemmae. However, the specific status of *A. colorata* was long debated because of the irregularity of the appearance of the papillate oogonia which were regarded by early investigators as the most important specific characteristic of this fungus.

A. colorata was first described by Cornu (1872) as *A. racemosa* var. *stelligera* and *A. racemosa* var. *spinosa.* Pringsheim (1882) renamed these varieties *A. colorata,* primarily on the basis of oogonial papillation. Fischer (1892), finding smooth and papillate oogonia on the same hyphae, considered *A. colorata* to be the same as *A. racemosa.* Minden (1912) continued to follow Cornu, describing in addition a form, *Pringsheimii,* of *A. racemosa* with "a few projections which may give the oogonia an angular appearance" and slightly fewer oospores. It remained for Coker (1923) to point out that "It is true that smooth or nearly smooth oogonia may appear rarely in this species, but they are not the oogonia of *A. racemosa,* which are always easily recognized by their much smaller eggs." The oospores of *A. racemosa* are mostly about 22 μ in diameter, those of *A. colorata* mostly 30–37 μ. Coker also states that "We have never seen a perfectly smooth oogonium in *A. colorata* or a papillate one in *A. racemosa.*" This has not been true of our cultures of *A. colorata.*

It is curious, since it has been known for so long that temperature affects not only quantitative, but also qualitative characteristics

of living organisms, that mycologists working with the Saprolegnia-
ceae, and with many other groups of fungi, have in the past paid so
little attention to the temperatures at which the cultures they have
described were grown.   The effect of temperature on papillation in
this fungus came to my attention more or less accidentally: a
mycelial mat (derived from a single-spore culture which had been
previously identified as *A. colorata* when grown on hemp seed at
15° C.) grown in pure culture at 25° C. produced apparently smooth
oogonia.   This culture was then incubated at 15° C. for two days,
during which period papillate oogonia appeared.

In order to determine more precisely the effect of temperature on
papillation in *A. colorata,* cultures were grown, on several media, at
temperatures ranging from 10 to 30° C. at 5° intervals.   No growth
occurred at 30° C. on any of the media used.   Three single-spore
isolates of *A. colorata,* from a collection of soil, made in Palisades
Interstate Park, N. J., were used in these experiments: in the later
experiments a culture derived from a single hyphal tip from an iso-
late obtained from Dr. Shanor was also employed as inoculum.

Unifungal cultures on boiled halves of popcorn and hemp seed
in petri dishes containing charcoal-treated distilled water gave some-
what equivocal results.   On both of these substrates oogonia were
invariably papillate at 10 and 15° C., though papillation was rather
more regular at the higher temperature.   The first oogonial initials
appeared in five to six days at 10° C., three days at 15° C.   Oogonia
appeared in two days in cultures incubated at 20° C., but when the
cultures were examined on the fifth day a minority of the oogonia
were atypical, with fewer, shorter papillae than at 15° C., or rarely
with the surface of the oogonium merely uneven.   This tendency
toward the suppression of papillae was marked in cultures grown
at 25° C., though even here only approximately half of the oogonia,
mainly those nearer the substrate, showed atypical characteristics
ranging from possession of only one or two papillae to complete
smoothness of the oogonial surface.

In order to minimize the effects of uncontrolled variable factors—
such as food and oxygen and bacterial contamination (which sooner
or later always became evident in the unifungal cultures)—*A.
colorata* was then grown in pure culture on both liquid and solid
media.   Difco corn meal agar to which 0.5 g./l. of yeast extract had
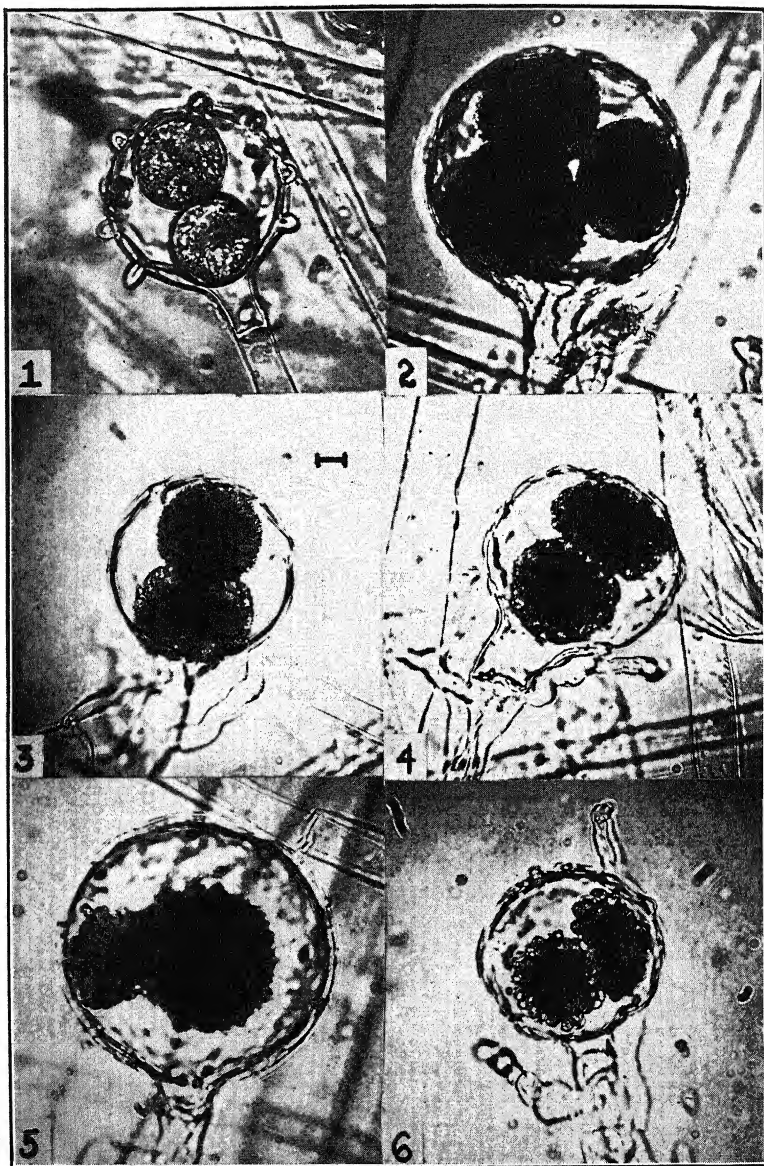
Figs. 1–6. Oogonia of *Achlya colorata*.

been added was used as the solid medium. Cultures grown on this agar in petri dishes gave the following results: At 10 and 15° C. papillate oogonia appeared in eight and four days respectively. At 20° C. the oogonia, appearing from the second day of growth, were usually roughened or with a few short papillae, infrequently reaching the extremes of normal papillation (as described for the species) or of complete smoothness of surface. At 25° C. the oogonia formed were roughened or completely smooth, never papillate, aborting or producing oospores which were nearly all aborted by the eighth day of growth. The position of the oogonia in the agar or on the surface of the agar did not obviously affect papillation.

The liquid medium employed consisted of 5 g./l. of yellow corn meal (as an extract made by boiling with distilled water ½ hour and centrifuged clear before use), 1 g./l. of yeast extract and 5 or 2.5 g./l. of glucose. There was no difference in papillation associated with the difference in concentration of glucose. *A. colorata* was grown in 20 ml. portions of this broth in 250 ml. Erlenmeyer flasks until the appearance of the first oogonial initials (at 7–10, 5, 3 and 3 days for cultures incubated at 10, 15, 20 and 25° C. respectively), when the mats were transferred, without washing, to sterile charcoal-treated distilled water in petri dishes. The mats matured more normally, though papillation was not markedly affected, when treated in this way than when allowed to remain in the culture broth. It was found that sexual reproduction was inhibited by washing the mycelia. Results were as follows: Oogonia were never formed in abundance at 10° C. and those formed were somewhat atypical and aborted. At 15° C. papillate oogonia were produced. An oogonium typical both of the cultures grown at 15° C. and of the species as described is shown in figure 1. The papillae, 6–10 μ long, are set regularly about the entire surface of the oogonium. The number of papillae may be slightly fewer than in this figure or sometimes greater, with occasional papillae touching at their bases or fused along their sides. The oogonia, irregularly set with short papillae, or merely roughened, shown in figures 2, 3 and 4, are typical of about 90 per cent of those appearing at 20° C. Oogonia typical of about the same percentage of those produced at 25° C. are shown in figures 5 and 6 as roughened or with 1 or

2 short papillae.   None was as papillate as any of the oogonia produced in the 15° C. cultures.   A completely smooth oogonium was rarely seen.   The oospores almost invariably aborted.

It seems likely that the differences of opinion regarding papillation of the oogonia of *A. colorata* have been caused by differences in temperature in the laboratories in which this fungus has been studied.   Pure cultures, grown under described conditions, should be used for the description of species.   The use of mixed or even of unifungal cultures may have contributed to the confused status of many species of the Saprolegniaceae and should be discouraged.

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EXPLANATION OF FIGURES

FIGS. 1–6.   Oogonia of *Achlya colorata* from pure cultures grown in broth.   × 400.   The photomicrographs were taken with a Leica Makam, using a 10 × ocular and a 44 × objective.   The line drawn on figure 3 indicates the relative length of 10 μ.   FIG. 1.   An oogonium from a mat incubated at 15° C.   FIGS. 2, 3 and 4.   Oogonia from mats incubated at 20° C. FIGS. 5 and 6.   Oogonia from a mat incubated at 25° C.   The oospores in both oogonia are already beginning to degenerate.

AMINO ACIDS IN THE BIOSYNTHESIS OF PENICILLIN

Frederick T. Wolf

Several investigations have been concerned with amino acids as sources of nitrogen for the production of penicillin. Foster *et al.* (1943) were apparently the first to test individual amino acids as sole sources of nitrogen for penicillin production by *Penicillium notatum* in surface cultures, finding that nitrate nitrogen was superior to amino acids. In subsequent experiments with submerged cultures, these workers (1946) noted that glutamic acid was superior to asparagine or glycine as a nitrogen source for one mold strain tested. With another strain, however, various single amino acids did not promote penicillin formation in the basal medium, either with or without additional sources of nitrogen.

Large increases in penicillin yield obtained in media containing corn steep liquor were found by White *et al.* (1945) to be at least partially due to arginine, histidine and glutamic acid. When a mixture of these compounds, in concentrations of 30 mg., 30 mg., and 400 mg. per 100 ml., respectively, was added to a basal medium containing glucose, lactose and mineral salts, yields assaying 80–90 per cent of that obtained in corn steep media resulted. This finding suggested that amino acids might be involved in the biosynthesis of penicillin: "It would appear that in some way this mold is perhaps able to convert one or several of the amino acids to a part of the penicillin molecule through its metabolic processes." It was further shown that hydrolyzed corn steep liquor is unsatisfactory for penicillin production, in comparison with hydrolyzed protein-free liquor, the large increase in free amino acids resulting in conditions unfavorable for penicillin formation.

Halpern *et al.* (1945) reported increased penicillin production upon the addition of proline or glutamic acid to synthetic media. Aspartic acid could be substituted for glutamic acid, and the addition of arginine also had a slight stimulatory effect. A number of

other amino acids, however, including histidine, methionine, trypto-
phane, hydroxyproline and phenylalanine, were ineffective.

Stone and Farrell (1946) failed to obtain consistent increases
in penicillin yield upon the addition of most of the common amino
acids to synthetic media, although occasional increases sometimes
followed the addition of leucine, cystine or cysteine. Cook and
Brown (1946) recorded increased yields upon the simultaneous
addition of glucose and leucine or glucose and tyrosine, although the
addition of the amino acids alone gave only a slight stimulatory
effect.

In a subsequent paper (1947) these same workers studied more
thoroughly the influence upon penicillin yield in surface cultures of
the addition of various amino acids to a basal medium containing
$NaNO_3$. The amino acids were added in such quantities as to
provide a nitrogen content (exclusive of $NaNO_3$) of 140 mg. per
cent. Under these conditions, small increases in penicillin yield
were obtained with *dl*-tyrosine, serine, cystine, *dl*-aspartic acid,
glutamic acid, arginine, histidine, *dl*-tryptophane, proline and as-
paragine. Glycine, alanine, valine, *d*-isoleucine, phenylalanine,
l-dihydroxyphenylalanine, methionine or *dl*-lysine, however, had no
stimulatory action on penicillin formation. No correlation be-
tween stimulatory effect upon penicillin yield and chemical struc-
ture of these compounds was possible.

Bonner (1947) tested alanine, serine, valine, cystine and methi-
onine as possible penicillin precursors in surface and submerged
cultures. Experiments using combinations of mutant "penicillin-
less" strains, to which these compounds were supplied singly, in the
presence of phenylacetic acid, and in the presence of *dl*-penicil-
lamine, yielded completely negative results.

Cardinal and Hedrick (1948) have recently presented analyses
of the amino acid content of corn steep liquor. A corn steep liquor
hydrolysate was found by microbiological assay to contain alanine
in excess of 27 per cent of the nitrogen present, large amounts of
arginine, glutamic acid, histidine, leucine, proline, lysine, valine,
threonine and isoleucine, as well as smaller quantities of phenyl-
alanine, aspartic acid, cystine, methionine and tyrosine.

The present study was undertaken because of the diversity of
results obtained concerning the role of various amino acids in the

biosynthesis of penicillin, resulting in part from the use of different mold strains and a variety of basal media by various workers. Further, no data of this type are available concerning *Penicillium chrysogenum* Q-176, the strain employed in almost all commercial penicillin production at the present time.

MATERIALS AND METHODS

P. chrysogenum Q-176 was employed in the present experiments. Stock cultures were maintained on slants of Czapek's agar. The basal medium for submerged cultures was adapted from one developed by Moyer and Coghill (1946) and has the following composition: lactose monohydrate, 27.5 g.; glucose monohydrate, 3.0 g.; $MgSO_4 \cdot 7H_2O$, 0.25 g.; KH_2PO_4, 0.50 g.; $ZnSO_4 \cdot 7H_2O$, 0.044 g.; and $MnSO_4 \cdot 4H_2O$, 0.020 g. per liter. To this basal medium were added various amino acids as sole sources of nitrogen, in quantities sufficient to provide a nitrogen concentration of 50 mg. per cent. Controls were provided in which the nitrogen was supplied as $NaNO_3$ (50 mg. per cent of nitrogen) or as corn steep solids (22.0 g. per liter). No phenylacetic acid or other penicillin precursor was added to the media; hence the penicillin produced, except in the series containing corn steep solids, was probably largely penicillin K (Higuchi *et al.,* 1946).

The cultures were grown at room temperature, in Erlenmeyer flasks of 500 ml. capacity containing 100 ml. of medium. The cultures were continuously agitated by means of a reciprocating shaker operating at the rate of 100 strokes per minute, with a stroke length of 7.0 cm.

All penicillin assays were performed on filtrates of cultures 7 days of age. The assay for total penicillins was carried out by means of the dilution technique, using *Staphylococcus aureus* H as the test organism. The broth from the *Penicillium* cultures was removed under sterile conditions, dilutions of 1 : 5, 1 : 25, 1 : 125, 1 : 625 and 1 : 3125 were made in tubes of Difco Heart Infusion Broth, and each tube was inoculated with 0.1 ml. of a 24 hr. culture of *S. aureus.* The tubes were incubated at 37° C. and read after 18 hrs. After determination of the highest dilution inhibiting growth and the lowest dilution with apparent turbidity, an aliquot of the penicillin broth, which had been stored in a refrigerator, was reassayed.

For the reassay, another series of five tubes was prepared, including the concentrations of penicillin immediately above and below the end point of the preliminary assay, with three intermediate concentrations interpolated. The highest dilutions inhibitory to growth of *S. aureus* are included in table I.

The sensitivity of *S. aureus* H, using commercial penicillin G as a standard, was determined to be 0.030 units/ml. This value was checked repeatedly during the course of the experiments, and, as no variation was found, it was employed in the conversion of dilution units to Oxford units.

RESULTS

Good growth of *P. chrysogenum* was obtained with each of the 24 amino acids tested, with the exception of cystine and cysteine.

TABLE I

PENICILLIN PRODUCTION BY *P. chrysogenum* Q-176 FROM
VARIOUS AMINO ACIDS

Amino Acid	mg./100 ml.	Max. Dilution Inhibitory to *S. aureus*	Penicillin Yield, Oxford units/ml.
Glycine	269	1:625, 1:625	19
dl-Alanine	318	1:375, 1:1250	24
beta-Alanine	318	1:1250, 1:3125, 1:625, 1:625	42
dl-Serine	376	1:125, 1:1250	20
dl-Threonine	427	1:250, 1:625	13
l-Arginine·HCl	188	1:125, 1:125	4
l-Aspartic acid	476	1:375, 1:375, 1:625	14
l-Asparagine	236	1:500, 1:625, 1:625	18
l-Glutamic acid	526	1:1250, 1:250, 1:625	21
l-Proline	410	1:625, 1:250, 1:250	11
l-Hydroxyproline	467	1:1250, 1:1250, 1:250	27
l-Leucine	467	1:25, 0, 1:25	0.50
dl-Isoleucine	467	1:5, 1:5, 1:5	0.15
dl-Norleucine	467	0, 0, 0	0
dl-Valine	420	1:5, 1:5, 0	0.10
l-Histidine·HCl	228	0, 1:5, 0	0.05
l-Lysine·HCl	327	0, 0, 0	0
l-Cystine[1]	431	0, 0, 0[2]	0
dl-Methionine	532	0, 0, 0	0
l-Cysteine·HCl	562	—, —, —[3]	0
l-Tryptophane	365	0, 0, 0	0
l-Tyrosine[1]	649	1:25, 1:25, 1:25	0.75
dl-Phenylalanine	588	0, 0, 0	0
NaNO₃	300	1:3125, 1:2500, 1:625, 1:2500	66
Corn steep solids	2200	1:6250, 1:6250	187

[1] Insoluble.   [2] Only a trace of growth.   [3] No growth, no assay made.

Only a trace of growth was obtained in media containing cystine. Microscopic examination showed that spore germination occurred, but mycelial growth was very restricted. This observation is in agreement with the finding (Wolf, **1948**) that cystine is not oxidized by this strain of *P. chrysogenum*. Cysteine monohydrochloride was evidently toxic in the concentration employed, as germination was completely inhibited.

Although no quantitative determinations of the amount of mycelial growth were made, it was apparent that proline and glutamic acid are particularly favorable for growth of *P. chrysogenum*. Alanine, which was previously shown (Wolf, **1948**) to be respired at a very rapid rate by this strain, did not support as luxuriant mycelial growth as did proline or glutamic acid.

In table I are included the various amino acids tested, the quantity of each which was used (equivalent to 50 mg. per cent of nitrogen) and the results of penicillin assays from 2–4 experiments with each amino acid.

DISCUSSION

In explanation of the apparently low yields obtained in cultures with $NaNO_3$ or corn steep solids as the nitrogen source, it should be borne in mind that the higher yields of commercial penicillin production employing this strain are obtained with media containing both $NaNO_3$ and corn steep solids, and supplemented by phenylacetic acid or other penicillin precursors. From the data presented it is evident that *P. chrysogenum* is able to achieve a total synthesis of penicillin from the basal medium when supplemented with certain amino acids as sole sources of nitrogen. These include several amino acids of low molecular weight, including glycine, alanine, beta-alanine, serine and threonine; the dicarboxylic compounds, glutamic acid and aspartic acid; asparagine; proline and hydroxyproline; and arginine.

The low values obtained with leucine, isoleucine, valine, histidine and tyrosine are believed not to be significant, in view of the possibility of the presence of small quantities of impurities in the amino acids used. Essentially negative results were thus obtained with cystine, cysteine, methionine, histidine, lysine, leucine, isoleucine, norleucine, valine, tryptophane, tyrosine and phenylalanine. The

results with cystine and cysteine are necessarily inconclusive, since mycelial growth is a prerequisite for penicillin formation.

No explanation is readily apparent for the finding that certain amino acids are able to participate in the biosynthesis of penicillin, while others cannot. The suitability of a given amino acid for penicillin formation cannot be correlated with its rate of oxidation by *P. chrysogenum* (Wolf, 1948), or with its suitability for mycelial growth. The fact that only amino acids with six or fewer carbon atoms are effective, and that a number of these are closely related structurally or metabolically, would suggest that the carbon skeleton may be important. It has recently been shown by Knight (1948) that *P. chrysogenum* Q-176 deaminates both alanine and methionine, liberating one molecule of ammonia for each atom of oxygen consumed. Therefore, penicillin formation from methionine is not blocked by failure of deamination to occur. This would suggest that the various keto-acids resulting from amino acid deamination might be most important in relation to penicillin biosynthesis.

Moderately good agreement was obtained with the findings of Cook and Brown (1947). Identical results were obtained in each case with serine, aspartic acid, glutamic acid, arginine, proline and asparagine, which take part in penicillin formation, and with valine, isoleucine, phenylalanine, methionine and lysine, which do not. The results do not agree in the case of tyrosine, cystine, histidine, tryptophane, glycine and alanine. Further, the present results are in agreement with those of Halpern *et al.* (1945), except in the case of hydroxyproline. The discrepancies noted are probably attributable to the use of different mold strains, which may well exhibit metabolic differences of this character.

None of the amino acids proved superior to nitrate as a nitrogen source for penicillin formation, thus confirming the finding of Foster *et al.* (1943) with surface strain of *P. notatum*.

In addition to other known functions of corn steep liquor in promoting penicillin formation, it would appear from the present data that the suitability of this material is due, at least in part, to its content of the amino acids usable in penicillin biosynthesis; namely, alanine, arginine, glutamic acid, proline, threonine, and aspartic

acid, which together, according to Cardinal and Hedrick (1948), make up about 54 per cent of its nitrogen content.

SUMMARY

When grown on a basal medium with the addition of single amino acids as sole sources of nitrogen in quantities equivalent to 50 mg. per cent of nitrogen, *Penicillium chrysogenum* Q-176 is able to produce small amounts of penicillin from glycine, alanine, beta-alanine, serine, threonine, arginine, aspartic acid, asparagine, glutamic acid, proline, and hydroxyproline. No penicillin is produced from leucine, isoleucine, norleucine, valine, histidine, lysine, cystine, methionine, cysteine, tryptophane, tyrosine, or phenylalanine under identical conditions. None of the amino acids tested is superior to nitrate as a source of nitrogen for penicillin biosynthesis.

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CHROMOSOME NUMBERS IN THE HYPOCREACEAE

Hilde E. Hirsch, William C. Snyder, and H. N. Hansen

(WITH 2 FIGURES)

The use of smear techniques (McClintock, 1945; Cutter, 1946) recently has advanced the study of chromosomes in the ascomycetous fungi. The present paper reports a brief survey, carried out with such methods, of the chromosome numbers of several species belonging to different genera of the family Hypocreaceae.

It has recently been shown that homothallic forms of *Hypomyces solani* Rke. et Berth. emend. Snyd. et Hans. (FIGS. 1F and 2F) have six chromosomes in the haploid stage (Hirsch, 1949 [1]), while the heterothallic *H. solani* f. *cucurbitae* Snyd. et Hans. may have four (FIGS. 1E and 2E), three or two chromosomes, depending on the sex of the isolate (Hirsch, 1947). It was therefore of interest to examine the chromosome number of the closely related genera *Gibberella, Calonectria* and *Nectria*. We have so far studied only species of these genera in which there is a conidial stage belonging to the imperfect genus *Fusarium*. The following were studied: *Gibberella roseum* Lk. emend. Snyd. et Hans. (homothallic), *G. lateritium* Nees emend. Snyd. et Hans. (heterothallic?), *Calonectria rigidiuscula* (Berk. et Brme.) Sacc. (homothallic), *Nectria episphaeria* f. *coccophila* (Desm.) Snyd. et Hans. (heterothallic?).

Perithecia were obtained in the first three fungi by growing them on water agar, to which was added ground up wheat straw sterilized by propylene oxide vapor (Hansen and Snyder, 1947), outside a north window. In the case of *Nectria* a small piece of a twig infested with scale insects, but also subjected to gaseous sterilization, was substituted for the wheat straw (Snyder and Hansen, 1947). The perithecia were fixed and smear preparations of the asci prepared and stained by the method referred to above.

[1] Hirsch, Hilde E. The cytogenetics of sex in *Hypomyces solani* f. *cucurbitae.* Amer. Jour. Bot. **36**: 113–121. 1949.

Both *Gibberella lateritium* and *G. roseum* (FIGS. 1A, 1B and 2A, 2B respectively) proved to have six pairs of chromosomes in the fusion nucleus. *Calonectria* (FIGS. 1C and 2C) and *Nectria* (FIGS. 1D and 2D), on the other hand, have seven.
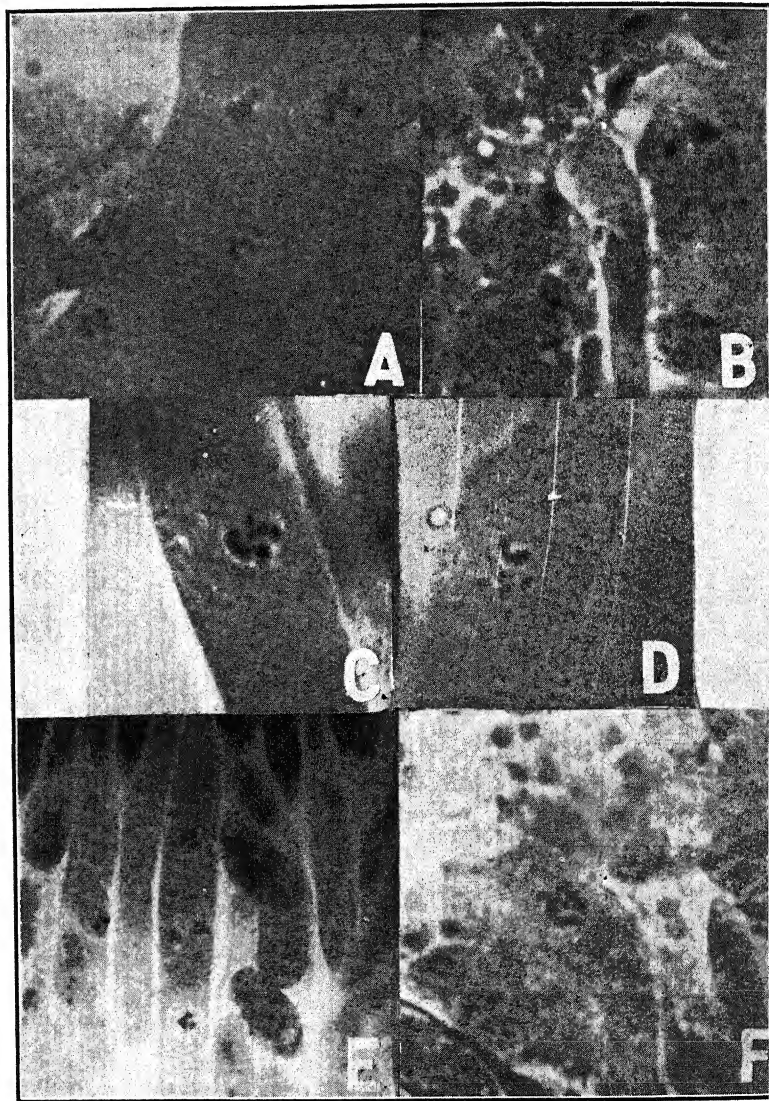
FIG. 1. Chromosome numbers in the Hypocreaceae.

No detailed study of the relative size and the morphology of the different chromosomes has been attempted so far. There is a remarkable uniformity in the cytology of all the species of Hypocreaceae studied by us, except for the *Nectria*. Here the asci are
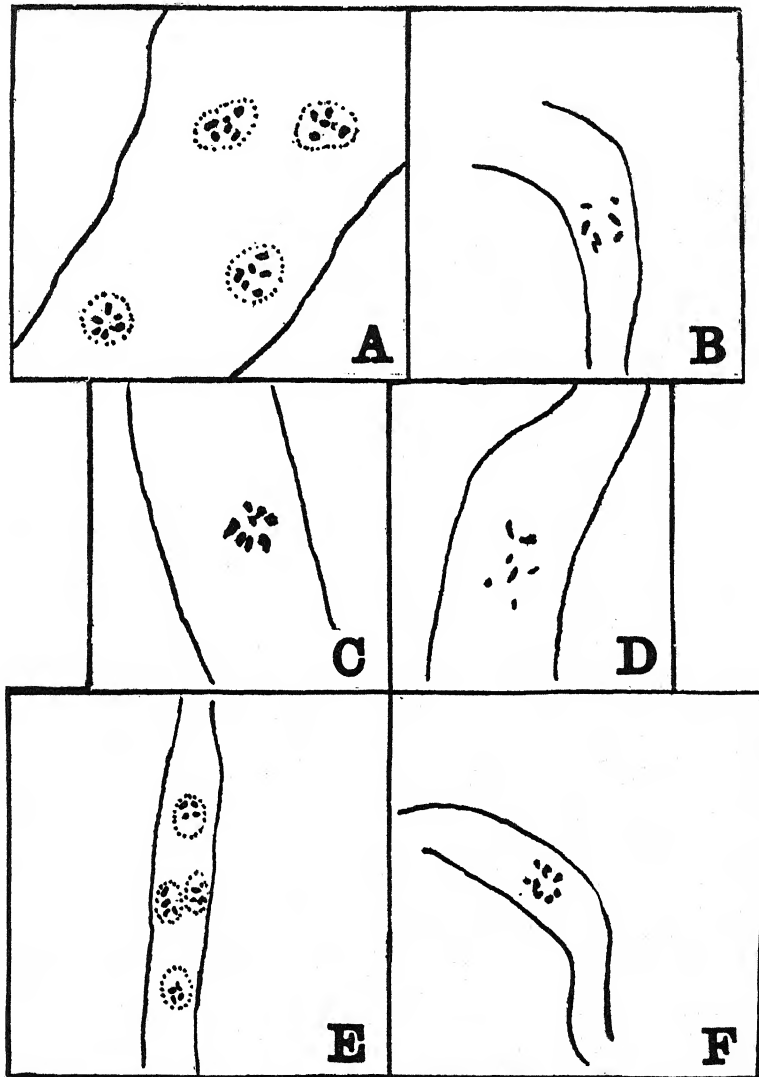
FIG. 2. Drawings interpreting fig. 1.

interspersed with sterile cells, and they react differently to stains. Although the other species were stained with Barrett's haematoxylin or acetocarmine, and could not be stained with the acetic orcein used, only the latter stain was effective on *Nectria*. There is also a remarkable difference in the size of the nucleolus (up to 4.5 μ in diameter during early meiotic prophase in *Nectria,* and up to 2.6 μ in *Hypomyces*).

It has been suggested (Snyder and Hansen, 1945) that the generic distinction between *Nectria* and *Hypomyces* is doubtful in those species which have *Fusarium* imperfects. Similarly, the validity of retaining both the genera *Gibberella* and *Calonectria* has been questioned. Although the difference in chromosome number would appear to suggest a possible basis for distinction between genera in these two cases, it must be remembered that different chromosome numbers frequently occur within a single species, as has been shown in *H. solani* (Hirsch, 1947). Much more needs to be known about the homologies of the different chromosomes before cytological observations can help us to form a clearer idea of taxonomic relationships. In this connection, species of *Nectria* and *Hypomyces* which have imperfect stages other than *Fusarium* will have to be studied.

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EXPLANATION OF FIGURES

Fig. 1. A–F. Chromosome numbers in *Gibberella, Calonectria* and *Nectria* species. A. *Gibberella lateritium.* Third metaphase, 6 chromosomes in each nucleus. (About 3,200 ×.) B. *Gibberella roseum.* Diakinesis, 6 chromosome pairs. (About 2,000 ×.) C. *Calonectria rigidiuscula.* Diakinesis, 7 chromosome pairs. (About 3,000 ×.) D. *Nectria episphaeria* f. *coccophila.* Second metaphase, 7 chromosomes. (About 2,600 ×.) E. *Hypomyces solani* f. *cucurbitae,* cross between two hermaphroditic strains. Second interphase, 4 chromosomes in each nucleus. (About 1,800 ×.) F. *H. solani;* homothallic form. Diakinesis, 6 chromosome pairs. (About 2,500 ×.)

Fig. 2. A–F. Interpretation of chromosome content of nuclei shown in figure 1. A. *Gibberella lateritium,* 6 chromosomes. B. *G. roseum,* 6 chromosome pairs. C. *Calonectria rigidiuscula,* 7 chromosome pairs. D. *Nectria episphaeria* f. *coccophila,* 7 chromosomes. E. *Hypomyces solani* f. *cucurbitae,* 4 chromosomes. F. *H. solani,* 6 chromosome pairs.

AN UNDESCRIBED SPECIES OF
PERICONIA

C. L. Lefebvre, A. G. Johnson, and Helen S. Sherwin [1]

(with 1 figure)

In September 1947, the senior writer collected at Garden City, Kansas, a plant of Colby milo that showed the characteristic symptoms of milo disease caused by *Periconia circinata* (Mang.) Sacc. as described by Leukel.[2] The following October, portions of the diseased roots of the above milo plant were placed in a petri-dish moist chamber for observation. After a few days, the characteristic *P. circinata* was evident at a number of points (FIG. 1, A). In addition, another somewhat similar fungus, very evidently a species of *Periconia,* was noticed, in some cases coming from the same rootlets that showed *P. circinata.* The conidiophores, however, were erect instead of being circinate as in *P. circinata,* and the conidia, although they were globose, were distinctly larger and their surfaces were very much rougher than those of *P. circinata* (FIG. 1, B–E).

Subsequently, single-spore cultures were made of the unidentified fungus and these were used in a soil inoculation experiment to test the pathogenicity of the fungus in comparison with *P. circinata.* Sand-cornmeal cultures of each fungus were mixed separately at two rates (16 oz. and 32 oz. per flat) with sterile soil and each lot of inoculated soil was placed in a different flat, $12 \times 24 \times 3$ inches. Two additional flats were filled with similar sterile soil for controls. To one of these, 16 oz. of the sterile medium was added, and to the other, 32 oz. Seed of susceptible and resistant Colby milos, as

[2] Leukel, R. W. *Periconia circinata* and its relation to milo disease. Jour. Agr. Res. 77: 201–222. 1948. *Illus.*

well as certain other grasses,[3] was sown in rows in all six flats and the flats kept in a warm greenhouse.

Periconia circinata attacked the susceptible but not the resistant milo, the same as reported by Leukel,[4] while the other grasses were not attacked.   After six weeks, in the soil inoculated at both rates with *P. circinata,* the susceptible milo plants ranged from 1 to 2 inches high while those of the resistant milo ranged from 20 to 21 inches.   The plants of both the susceptible and the resistant milos in the control flats averaged about 20 inches high.

The unidentified species of *Periconia* attacked neither of the milos nor any of the other grasses.   In the soil inoculated at both rates with the unidentified species of *Periconia,* the plants of both the susceptible and resistant milos averaged about 20 inches high and were comparable with the controls in vigor and color.

It is evident, therefore, that the unidentified species of *Periconia* differs both morphologically and pathogenically from *P. circinata.* It likewise differs distinctly from the rough, globose-spored species of *Periconia,* listed by Linder.[5]   It differs distinctly also from *P. heveae* Stevenson and Imle [6] in a number of respects, particularly in the prominent, conical spines or papillae on the walls of the conidia.   Because of these various differences, the above unidentified species of *Periconia* is here proposed as:

Periconia macrospinosa Lefebvre et A. G. Johnson sp. nov.

Conidiophoris simplicibus vel apice sparse ramosis, rectis vel subflexuosis, totis atro-brunneis, 1–4-septatis (in cultura usque 11-septatis), usque circa 350 μ longis (in cultura longioribus), apud basim 10–11 μ in diam., supra 8–9 μ, cellulis basalibus et apicalibus paulo inflatis; *cellulis primariis sporogenis* pallide brunneis, forma variabilibus, 5–8 × 8–13 μ, circa cellulam apicalem verticellatis; *cellulis secondariis sporogenis* pallide brunneis, sphericis

[3] *Agropyron cristatum* (L.) Gaertn., *Andropogon scoparius* Michx., *Bouteloua curtipendula* (Michx.) Torr., *B. gracilis* (H.B.K.) Lag., *Buchloë dactyloides* (Nutt.) Engelm., *Eragrostis curvula* (Schrad.) Nees, *E. trichodes* (Nutt.) Wood, *Panicum antidotale* Retz., *P. virgatum* L., and *Stipa viridula* Trin.

[4] Loc. cit.

[5] Linder, David H.   New Venezuelan Fungi Imperfecti. Mycologia **29**: 656–664. 1937. *Illus.*

[6] Stevenson, J. A., and Imle, E. P.   *Periconia* blight of *Hevea.* Mycologia **37**: 576–581. 1945. *Illus.*
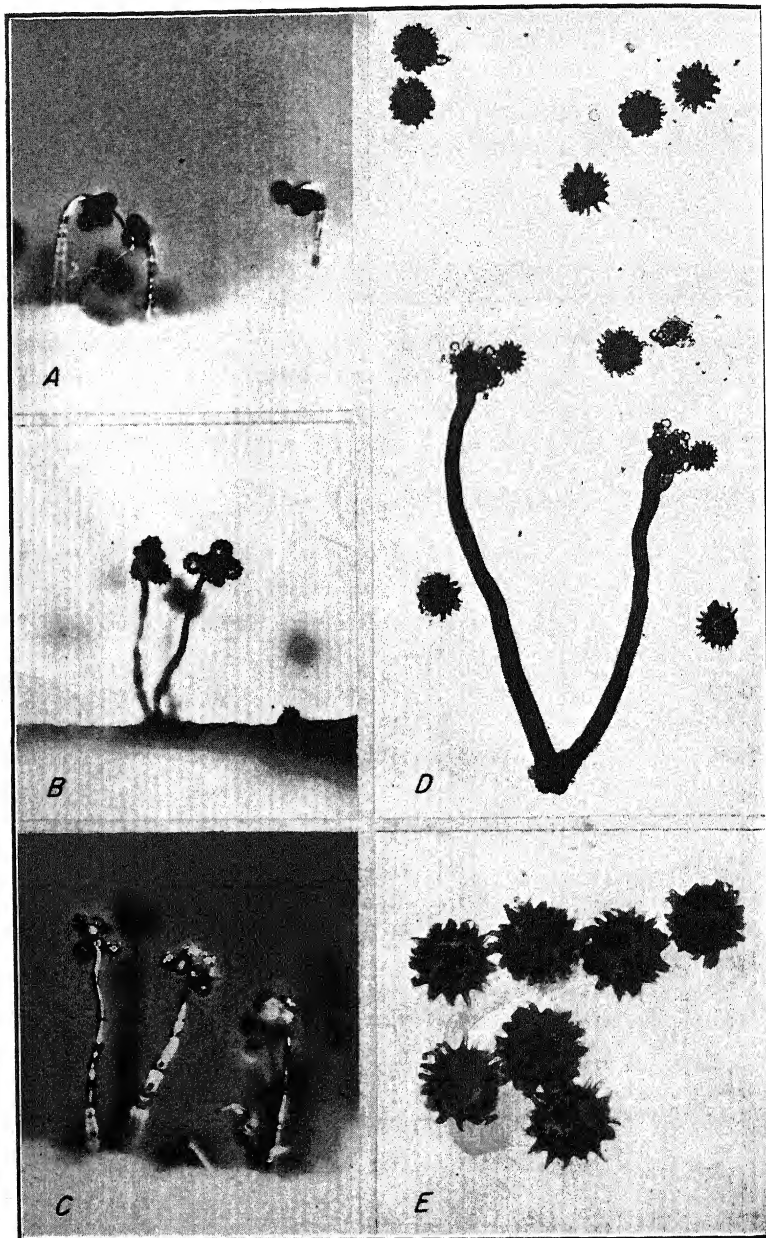
Fig. 1. *Periconia circinata* and *P. macrospinosa*.

usque late ovoideis, inconspicue verruculosis, 5–8 × 6–8 μ, in cellulis sporogenis primariis vel in cellula apicali conidiophori supra verticellum cellularum primariarum sporogenarum productis; *conidiis* atrobrunneis, sphericis, crasse spinosis, 18–32 μ, singulatim vel in catenulis brevibus fragilibus in cellulis secondariis sporogenis oriundis; *spinis* atro-brunneis, circa conicis, paulo deciduis, 2.5–6 μ altis, basi 2–3 μ in diam.

In radicibus emortuis *Sorghi vulgaris,* Garden City, Kansas, U. S. A.

Conidiophores simple or sparsely branched at tip, straight or slightly flexuose, dark brown throughout, 1–4-septate (up to 11-septate in culture), up to about 350 μ long (longer in culture), 10 to 11 μ in diameter near base, 8 to 9 μ above, the basal and apical cells somewhat enlarged; primary sporogenous cells light brown, variable in shape, 5–8 × 8–13 μ, forming a whorl about the apical cell; the secondary sporogenous cells light brown, spherical to broadly ovoid, inconspicuously verruculose, 5–8 × 6–8 μ, produced on the primary sporogenous cells or on the apical cell of the conidiophore above the whorl of primary sporogenous cells; conidia dark brown to black, spherical, coarsely spinose, 18–32 μ, borne singly or in short, fragile chains on the secondary sporogenous cells; spines dark brown, approximately conical, somewhat deciduous, 2.5–6 μ high, 2–3 μ in diameter at base.

On dead roots of *Sorghum vulgare* Pers. var. Colby milo, culture from specimen collected by C. L. Lefebvre, Garden City, Kansas, September 25, 1947 (**type**), deposited in the Mycological Collections of the Bureau of Plant Industry, Beltsville, Maryland, and in the Farlow Herbarium, Harvard University, Cambridge, Massachusetts.

BUREAU OF PLANT INDUSTRY STATION,
    BELTSVILLE, MARYLAND

EXPLANATION OF FIGURES

FIG. 1.   A, *Periconia circinata* showing circinate conidiophores and dark-colored conidia, × 120.   B–E, *P. macrospinosa:* B and C, straight to slightly flexuose conidiophores and dark-colored conidia, × 120; D, conidiophores, spinose conidia, two detached secondary sporogenous cells (near top), and detached spines, × 250; E, spinose conidia, × 500.   (A and C, photographed chiefly by reflected light, others chiefly or entirely by transmitted light.)

A CYTOLOGICAL STUDY OF TYPICAL AND ATYPICAL BASIDIAL DEVELOPMENT IN GYMNOSPORANGIUM CLAVIPES

Lindsay S. Olive

(with 14 figures)

In an earlier paper (1941), the writer described a procedure for germinating teliospores of *Gymnosporangium* on slides and killing and staining the material *in situ*. Heidenhain's haematoxylin was used to stain the nuclei. With this method, distinct black nuclei are observed against a light cytoplasmic background. Although the technique does not bring out details of nuclear structure, certain other important features may be observed, such as the numbers of nuclei in basidial cells and basidiospores, nuclear divisions, and the appearance of the nuclei during migration.

Recently, a graduate student of this department, Mr. Robert Carroll, observed during a study of some slides of *G. clavipes* prepared by the writer that one or two of the basidiospores which he encountered were quadrinucleate instead of binucleate and that these spores were somewhat larger than the normal binucleate ones. This led to the present study by the writer to determine how such spores are produced and what their significance in the life history of the rust might be.

The writer is very grateful to Dr. C. W. Edgerton for his invaluable assistance with the photographic work.

INVESTIGATIONS

The reader is undoubtedly already familiar with the cytological details in the development of a typical rust basidium. Nevertheless, for the purpose of comparison with atypical developments, the process will be briefly reviewed here.

The teliospores of *G. clavipes* are two-celled and each cell, upon germination, produces a basidium. The basidium is produced

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through a pore in the wall at the end of the teliospore cell. A portion of the cytoplasm passes out into the developing basidium and then the nucleus enters, becoming elongated and somewhat constricted as it passes through the region of the pore (FIG. 1). Eventually, the entire protoplasmic contents enter the young basidium, leaving the teliospore cell empty. The nucleus retains its elongated form (FIG. 1) and soon undergoes the first of the two divisions which are characteristic of basidial development. These are considered to be meiotic divisions.

With the present technique the prophase nuclei are generally stained a solid black (FIG. 1) and it is therefore impossible to distinguish the prophase chromosomes. However, during actual nuclear division, a distinct spindle and partially outlined chromosomes may frequently be observed (FIGS. 2, 3). The interphase between the first and second meiotic divisions is very short and the daughter nuclei enter almost immediately into the second division (FIG. 4). At this point, it is most important to observe the timing between cross wall formation and nuclear division. The first cross wall does not generally begin to appear until the second meiotic division is in progress. Occasionally it is not evident until after the second division has been completed. The septum appears about midway across the basidium. This is quickly followed by the appearance of a septum between the components of each pair of daughter nuclei of the second meiotic division, and a four-celled, four-nucleate basidium is produced (FIGS. 4, 7).

The basidium now enters into the final phase of development—the production of the basidiospores. Each cell gives rise to a sterigma which becomes quite pointed at its distal end. This pointed tip then expands into a subglobose structure which is the developing basidiospore. The cytoplasm and nucleus migrate through the sterigma into the swelling, the nucleus becoming much constricted as it passes through the attenuated portion of the sterigma (FIG. 5). Very soon after the nucleus has entered the developing basidiospore, it undergoes a mitotic division, the third in this series of nuclear divisions, and a binucleate basidiospore is produced (FIG. 6). This division is generally completed even before all of the cytoplasm has passed through the sterigma into the spore. The basidiospore is
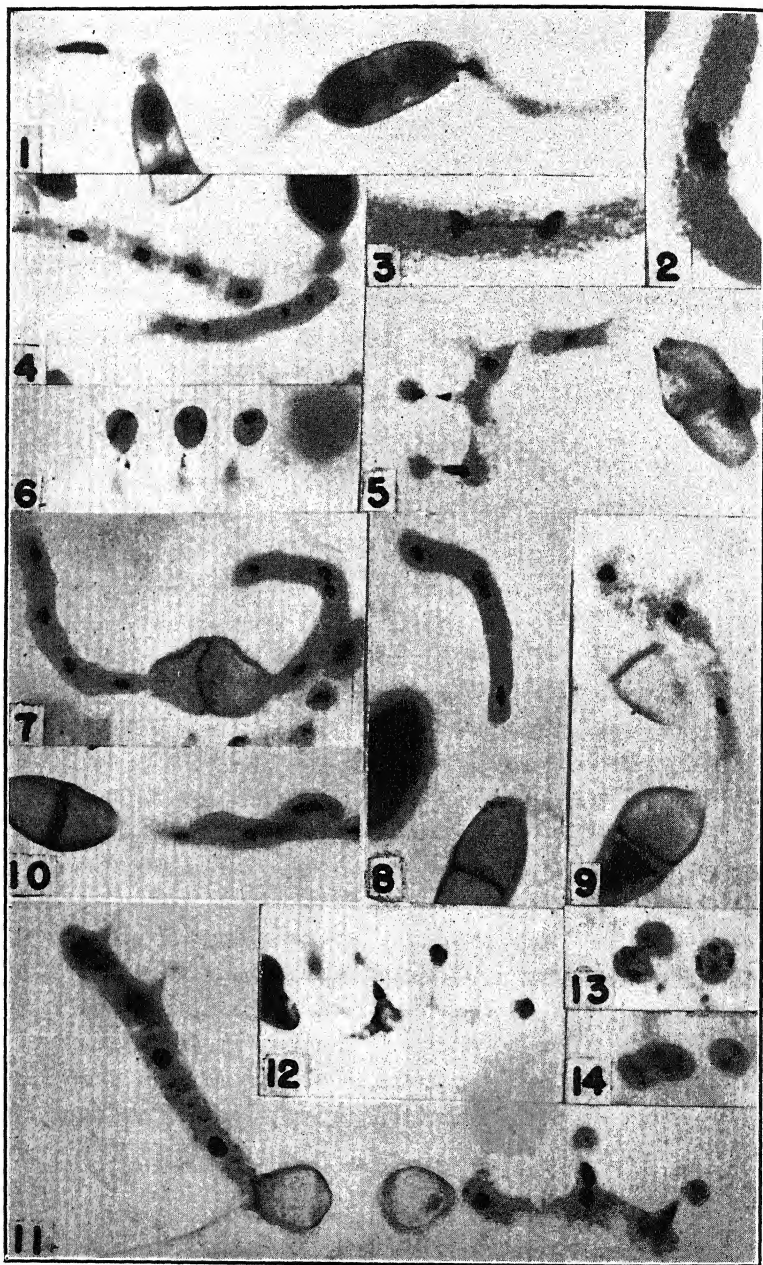
Figs. 1–14. *Gymnosporangium clavipes.*

discharged in the binucleate condition. Each basidium produces four binucleate basidiospores. The spores are not all to be found at the same stage of development on any one basidium. Frequently all stages of development may be observed on the same basidium. Generally the basidial cell nearest the teliospore is the last to produce a basidiospore.

Not all of the basidia of this rust are four-celled. A small proportion of them are three-celled. In such atypical basidia one cell contains two nuclei and each of the other two cells contains a single nucleus. The explanation for the occurrence of such basidia is to be found in a study of the relationship of cross wall formation to nuclear division. In most cases it is the middle cell of the three-celled basidium which is binucleate (FIGS. 7–9, 11). It has already been pointed out above that in the normal course of events the first cross wall in the basidium does not generally appear until the second meiotic division is in progress, or occasionally not until the division has been completed. In a few cases the first septum fails altogether to appear, while the two second division septa develop normally. This produces a basidium with a uninucleate cell at each end and a binucleate cell in the middle. Furthermore, the middle cell contains a nucleus from each of the second division spindles. There is no overlapping of spindles in the basidium.

Very rarely a three-celled basidium is found in which either the proximal or the distal cell is binucleate and the other two are uninucleate (FIGS. 10, 12). Two basidia of each type were observed during this study. In such basidia as these, it is apparent that the first septum developed normally, but one of the two second division septa must have failed to appear. The binucleate cell in such a basidium would obviously contain a pair of daughter nuclei derived from a spindle of the second meiotic division.

The three-celled basidium continues its development by producing three basidiospores instead of four. Each uninucleate cell gives rise to a normal basidiospore, while the binucleate cell gives rise to a basidiospore into which both nuclei migrate (FIGS. 11, 12). These two nuclei then undergo a mitotic division and a quadrinucleate basidiospore is produced (FIGS. 13, 14). These quadrinucleate basidiospores are conspicuously larger than the normal binucleate ones, as the photographs show.

An attempt was made to determine approximately what percentage of the mature basidia are three-celled. Well over a thousand basidia were examined to obtain this estimate. From this study it appears that about 0.75 per cent of the basidia are of the three-celled type.

<div align="center">DISCUSSION</div>

There occur in the literature on rust cytology several references to certain rusts which normally produce basidia with one or more binucleate cells, and these cells produce basidiospores that are binucleate at their origin. Lindfors (1924) studied a race of *Puccinia arenariae* which produces two-celled basidia that give rise to two binucleate basidiospores. These spores in turn produce binucleate mycelia upon infecting the host. Thirumalachar (1945) found in *Cystopsora oleae* two types of basidia—one, the typical four-celled basidium, and the other, a two-celled basidium with binucleate cells. The two-celled basidium produces two binucleate spores. Thirumalachar further claims that the "diplophase" (dikaryophase) is initiated in the basidiospore and that the basidiospore, upon infection of the host, gives rise to a "diploid" (dikaryotic) mycelium. This concept is probably correct, although verification with experiments in monosporidial infection is needed.

Recently the writer (1947), in a cytological study of basidial development in *Sphenospora kevorkianii,* found that this rust typically produces three-celled basidia. One of the cells, usually the middle one, is binucleate and produces a binucleate basidiospore. The other two cells, which are uninucleate, produce uninucleate basidiospores. It is considered likely that the binucleate spores, at least in the majority of cases, are heterokaryotic, but verification of this is lacking.

In *Gymnosporangium clavipes,* the basidium is normally four-celled and produces four basidiospores that are uninucleate at their origin. However, a mitotic division occurs just before the spore is discharged so that each spore normally contains two nuclei at maturity. Such a condition is common in the rusts and many other heterobasidiomycetes and it does not mean that the spore is heterokaryotic. On the contrary, these two nuclei are probably genetically

alike. The basidiospore produced by the binucleate cell of the atypical basidium, however, is binucleate at its origin and becomes four-nucleate by a mitotic division of each nucleus before spore discharge. It seems likely that the majority of these spores are heterokaryotic. It is now generally acknowledged that segregation of factors may occur in both meiotic divisions in the basidium. If segregation of compatibility factors should occur in the first division, then the central binucleate cell, which receives a nucleus from each of the second division spindles, would be heterokaryotic for the compatibility factors. If segregation of these factors occurs in the second meiotic division, the spore has an equal chance of being heterokaryotic or homokaryotic for the compatibility factors.

Infection experiments with these atypical basidiospores should prove quite interesting. Although, in *G. clavipes,* the percentage of such spores is small, they could probably be distinguished in nature from the binucleate spores by their larger size. Also it should be possible to locate some of the three-celled basidia before they discharge their spores. One might suspect that the atypical spore would be capable of infecting the rosaceous host and could produce a dikaryotic mycelium that would give rise to aecia. Of course there is the other possibility, less likely, that the spore might infect the gymnosperm host, *Juniperus virginiana.*

A careful study of other species of rusts which normally produce four-celled basidia would probably reveal the presence of a certain percentage of these atypical basidia and basidiospores. There is some indirect evidence in the literature which lends support to this belief. There are numbers of instances in which an investigator, while working with monosporidial infections in heterothallic rusts, has occasionally obtained an infection which produced aecia. In several cases such results have been marked off to poor technique or to contaminations. In other instances the investigator has suggested that the dikaryon may have arisen spontaneously in the mycelium within the host. In view of the present findings, the writer believes that such results may have been obtained, in a number of cases, through the use of heterokaryotic basidiospores. Further investigations are needed to determine if this theory is correct.

SUMMARY

In *Gymnosporangium clavipes* the basidia are normally four-celled and four-nucleate and each cell gives rise to a basidiospore which is uninucleate at its origin, but becomes binucleate before discharge by means of a mitotic division of its nucleus. A small percentage of the basidia are three-celled. One of the cells of the atypical basidium is binucleate and the other two are uninucleate. The binucleate cell gives rise to a basidiospore which is at first binucleate but which becomes four-nucleate as a result of mitotic divisions. The four-nucleate basidiospore is believed to be in the majority of cases heterokaryotic. The possible role of this type of spore in the life cycle of the rust is discussed.

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EXPLANATION OF FIGURES

FIGS. 1–14. *Gymnosporangium clavipes*. 1, migration of the fusion nucleus into the developing basidium; 2, anaphase of the first meiotic division; 3, telophase of first division; 4, two basidia, one showing telophase spindles of the second meiotic division, the other being a mature 4-celled basidium which is producing sterigmata; 5, normal 4-celled basidium producing basidiospores; 6, basidiospores showing stages in nuclear division before spore discharge; 7, teliospore with normal 4-celled basidium and atypical 3-celled basidium, the latter with the middle cell binucleate; 8, 9, two 3-celled basidia, each with a binucleate cell in the center; 10, a 3-celled basidium with terminal cell binucleate; 11, teliospore with a 4-celled and a 3-celled basidium, the latter producing basidiospores; 12, a 3-celled basidium with the proximal cell binucleate, basidiospores developing; 13, 14, binucleate and 4-nucleate basidiospores. (All photographs approximately × 500, except 2 and 3 × 1,175.)

THE TAXONOMY OF SEPTOBASIDIUM POLYPODII AND S. ALBUM

J. N. Couch

(WITH 25 FIGURES)

Several members of the Auriculariales have been described as parasitic on mosses and ferns. These belong in the genera *Jola, Eocronartium,* and *Herpobasidium,* the first two occurring on mosses and the last on a fern. In 1929 the writer described as *Septobasidium Polypodii* a new fungus parasitic on the sori of *Polypodium* sp. Since the vegetative structure was not too unlike that of certain species of *Septobasidium* and the probasidia and basidia were quite similar to those of that genus, it was classified as a *Septobasidium* in spite of the absence of scale insects beneath the stroma. Later studies by Boedijn and Steinmann (1931) and the writer (1938) have shown that all species of *Septobasidium* live with scale insects. On the basis of this very distinctive feature, *S. Polypodii* has been excluded from the genus *Septobasidium.* In 1939 the late Dr. Linder sent me a fungus which was growing as a parasite on the lower side of the leaflets of a fern. The resupinate habit, the probasidia and thrice-septate basidia suggested a relationship with the fungus formerly described as *Septobasidium Polypodii.* It is the object of this paper to give descriptions of these two species on ferns, to discuss their taxonomic positions, and also to present new observations on *Septobasidium album,* showing that it should be transferred to the genus *Helicogloea.*

The two species on fern would seem to be distinct enough from *Jola* and *Eocronartium* on mosses and from *Herpobasidium* on fern to justify the establishment of a new genus. Since the fruit body is flattened it is being called *Platycarpa.*

Platycarpa gen. nov.

Fructificatio resupinata, minima sicca vel subcartilaginea, facile separabilis ab hospite maturitate; hyphis non nodosis ad septa; hymenio levi; probasidiis

hyalinis, membranis incrassatis; basidiis cylindricis vel curvatis; sporis allantoideis.

Parasitica in foliis filicis; haustoriis robustis praesentibus vel absentibus.

Fruit body resupinate, very small, dry to subcartilaginous, easily separable from host at maturity; hyphae without clamp connections; hymenium smooth; probasidia hyaline with thickened walls; basidia cylindric or curved; spores bent-ellipsoid.

Parasitic on fern leaves or sporophylls; with or without highly developed haustoria. Intermediate between *Septobasidium* and *Iola*. Distinguished by its occurrence on ferns, its small, flattened fruit body and the absence of scale insects. In its tenuous connection with its host, *Platycarpa* is quite like *Septobasidium;* but the host in the former is the fern and in the latter, scale insects.

Platycarpa Polypodii n. comb.

Septobasidium Polypodii Couch. Jour. Elisha Mitchell Sci. Soc. 44: 255. 1929.

Resupinate, on the under surface of fertile fronds of *Polypodium* sp., parasitizing the sori and usually occurring in small patches up to 2 mm. wide, each patch limited to one fertile leaflet; sometimes the patches are confluent along the midrib of a sporophyll and then completely cover the lower surface for several centimeters; white when fresh, becoming cream-colored upon drying; in section 250–700 μ thick, usually about 500 μ thick, whitish throughout; composed of (1) a very thin lower layer of hyphae which extend over the lower surface of the fern leaf but do not penetrate into the epidermal tissue, and (2) a layer of compact, much-branched, coiled and entangled hyphae arising from the lower layer, often stratose by the successive formation of two or three fruiting regions; hyphae of context 3.8–5.4 μ thick, thick-walled, with inconspicuous septa spaced at irregular intervals, hyphae breaking up into short segments when crushed gently under a cover glass, noticeably constricted at the base of branches; probasidia formed near the outer surface of the compact layer, oval, 12.5–14.8 × 16–23 μ, wall slightly thickened, sometimes thicker on one side, germinating into a 4-celled, curved basidium 8.2–12.6 × 50–70 μ, with sterigmata about 8 μ long, each cell forming a spore; spores bent-ellipsoid, 6.5–8.5 × 19–25 μ, becoming once-septate, germinating by repetition; haustoria in shape of irregular coils within prosporangial and sporangial cells of host.

For a comparison of this species with *P. boliviensis* see that species.

Specimens examined:

Jamaica: Blue Mountains, on *Polypodium* sp., W. R. Maxon, coll., June 1926. **Type** in University of North Carolina Herbarium, and distributed to several other herbaria as *Septobasidium Polypodii.*
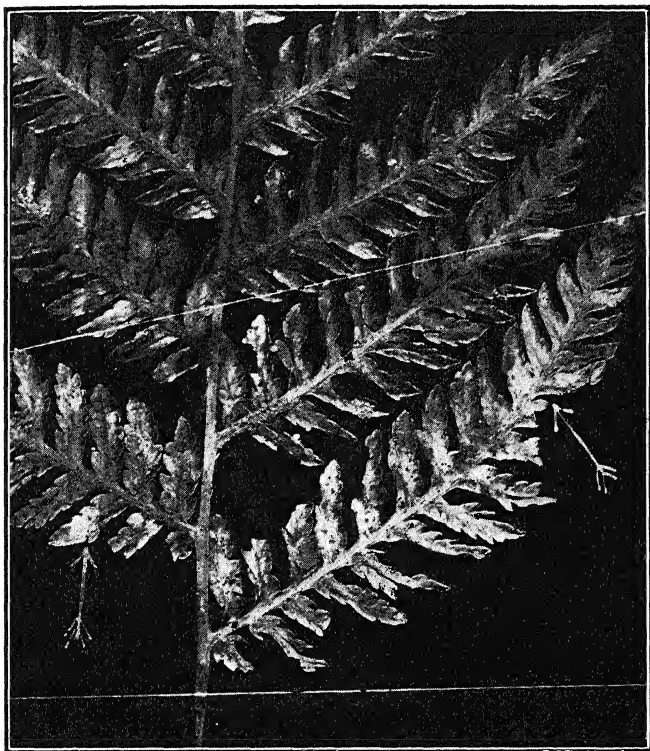
Fig. 1. Part of lower surface of dried fern leaf showing patches of *Platycarpa boliviensis.* These are usually lighter in color than leaf, as shown in small patches on left to which arrows point. On right, arrow points to area where fungus covers several leaflets.   × 2.

Platycarpa boliviensis n. sp.

Fructificatio resupinata, hypophylla, plerumque orbiculata, facile separabilis, circa 1–2 mm. lata, interdum extensa secundum mediam venam ad 10 mm.; 0.2–0.8 mm. crassa si humida; immatura fulva, matura argillacea vel fusca, partibus vetustis humidis cartilagineis; haustoriis incompositis massis in cellulis epidermidis et mesophyllis; hyphis laxe intertextis, ascendentibus,

hyalinis, rare septatis, ramosis, 4–5 μ in diametro; hymenio non valde distincto ex contexto; probasidiis pyriformibus, proliferatione interna, 16–25 × 25–30 μ, membranis leviter incrassatis, vacuis ex germinatione; basidiis deciduis, divisis in quatuor cellulas, cylindricis vel curvatis, 8–10.9 × 65–85 μ; sterigmatibus brevibus 6–10 μ; sporis 6.3–8.4 × 29–39 μ; hyphis hymenii inaequaliter curvatis, attenuatis, circa 2.2 μ in diametro.

Resupinate, hypophyllous, forming closely applied but very easily separable, thin (when dry), inconspicuous, somewhat circular or irregular patches, usually one patch to a leaflet, or sometimes covering the lower surface of several leaflets; circular patches 1 or 2 mm. wide, the larger patches extending along the veins for as much as 10 mm. Pale buff and pulverulent when young; clay colored or wood brown and glabrous when older and cartilaginous when wet. Loosely attached to the leaf by a comparatively few threads which penetrate through the epidermis and stomata and form very irregular masses of hyphae in the epidermal and mesophyll cells. In section 200–800 μ thick when moistened, composed of rather loosely entangled, hyaline hyphae which are rather sparingly septate and usually slightly constricted at the septa, non-nodose, twisted, thin- to rather thick-walled, branched and 4–5 μ thick. Hymenial region not sharply set off from the context. Probasidia hyaline, pyriform, 16–25 × 25–30 μ, walls slightly thicker than hyphal walls, the basal part and also the supporting hypha crooked; renewed by internal proliferation, sometimes as many as four old walls and a new probasidium on the end of the same stalk; germinating and becoming empty to form the basidia, which are deciduous and apparently collapse readily. Basidia 4-celled, cylindrical or irregularly curved, 8–10.9 × 65–85 μ (only a few good ones seen). Sterigmata short, *i.e.*, about 6–10 μ. Spores hyaline, bent-ellipsoid, 6.3–8.4 × 29–39 μ. Hyphae of hymenium irregularly curved, the ends of uneven diameter, usually about 2.2 μ thick.

This fungus may be distinguished by its parasitism on the fertile leaves of a fern but not on the sporangia, by the very thin membranous appearance of the fructification when dry, the cartilaginous texture when wet, the hyaline, sparingly septate hyphae without clamp connections, the large, internally proliferating probasidia, the 4-celled, straight, or slightly curved basidia, and the large, bent-ellipsoid spores.

The nourishing hyphae of this species develop irregular masses chiefly in the mesophyll cells, being only occasionally found in the epidermis, and thus differing rather strikingly from *P. Polypodii*

which has distinctly coiled haustoria within the cells of the very young sporangia.   The two species also differ in texture and in the shape and size of the probasidia, basidia, and spores.

The early stages of infection have not been observed in either of the species of *Platycarpa*.

Specimens examined:

Bolivia: On fern sporophylls, H. H. Rusby, coll., sent by D. H. Linder.   **Type** in Farlow Herbarium and University of North Carolina Herbarium.

Determining the relationships of the present genus offers considerable difficulty.   At one time the writer (**1938**) suggested a relationship between *Platycarpa* (*Septobasidium*) *Polypodii* and the very peculiar genus *Goplana,* which is considered by Raciborski (**1900**) as intermediate between the Uredinales and Auriculariales. *Goplana* now seems to the writer to be closer to the former than to the latter order since its hymenium lacks hyphal tissue intermingled with the basidia.   In *Platycarpa* the fructification consists of a comparatively large amount of sterile hyphal tissue, near the top surface of which is formed the hymenial layer composed of sterile hyphae, probasidia, and basidia, much as in certain species of *Septobasidium*.   The present genus can be distinguished from *Septobasidium* by the invariable association of the latter with scale insects.   Of all members of the Auriculariales it would seem to be closest to *Jola* and *Eocronartium,* both parasitic on mosses.   *Jola* and *Platycarpa* can be distinguished by habitat, the former occurring on mosses, the latter on ferns.   Also in *Jola* the probasidia are thin-walled and are formed in clusters, while in *Platycarpa* the probasidial walls are somewhat thickened and these structures are formed singly.   *Eocronartium,* an obligate parasite on mosses, seems closely related to *Jola,* a suggestion further confirmed by the recent discovery by Stanley (**1940**) and others of probasidial cells in *Eocronartium*.   This genus is readily distinguished from *Platycarpa* by the fact that the former occurs on mosses and by the probasidia and basidia.   In *Eocronartium* (perhaps also *Jola*) the hyphae are thin-walled, much septate, and apparently only one kind of hypha is formed.   In *Platycarpa* the hyphae are thin- to thick-
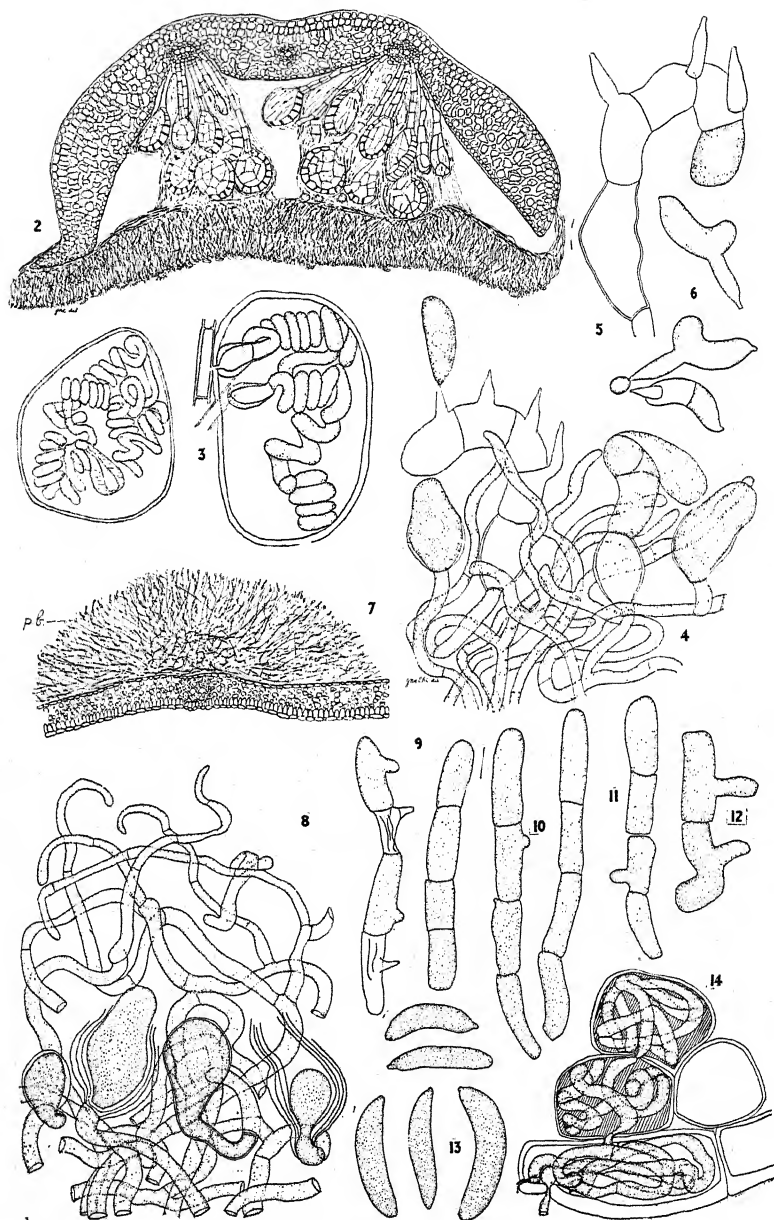
Figs. 2–14. *Platycarpa Polypodii* and *P. boliviensis.*

walled with rather infrequent septations and the hyphal system is
more complex.   Another difference between the two is in the ex-
tent of the nourishing hyphae.   In *Eocronartium* the hyphae ex-
tend through the stems and many of the leaves of the parasitized
gametophytic thallus (Fitzpatrick, **1918**), while in *Platycarpa* the
nourishing hyphae are quite limited in extent.   The genus *Cysto-
basidium* was transferred to *Jola* by Patouillard (**1900**) but it seems
best to me to follow Gäumann (**1922**) and treat it as a distinct
genus.   Both species of *Cystobasidium* have been reported as over-
growing other fungi, both lack hymenia, and both have clamp con-
nections; all of these are characters which readily distinguish
Cystobasidium from *Jola* and also from *Platycarpa*.

The genus *Herpobasidium* described by Lind (**1908**) and stud-
ied by Jackson (**1935**) occurs on ferns as an obligate systemic para-
site and must be compared with *Platycarpa*.   The following is taken
largely from Lind and Jackson with some additional notes made
from an abundance of dried material furnished me by Dr. Jackson.
When mature the diseased areas show on the upper leaf surfaces
as small brownish spots.   On the under surface of such areas a
white, mould-like growth is visible.   Sections of these spots show
abundant intercellular mycelium and conspicuous coiled intra-
cellular haustoria, usually in the mesophyll but also in the epidermal
cells.   Branches from the intercellular mycelium emerge in fascicles
through the stomata and these threads continue to branch and
spread over the lower leaf surface, forming a very thin (8–$34\,\mu$
thick) covering in the material examined by me.   (Lind gave the
thickness as 1 mm., width as 2 mm. and length as 4 mm.)   The
basidia arise from these horizontal hyphae, but in thin sections
these hyphae are so sparse and inconspicuous that about all one can
see are the basidia.   As nearly as I could make out there are no
sterile hyphae intermingled with the basidia in *Herpobasidium*, in
sharp contrast to *Platycarpa* in which there is a fruit body com-
posed of a relatively large amount of sterile tissue and relatively few
probasidia and basidia.   The coiled haustoria in *Herpobasidium*
and in *Platycarpa Polypodii* are quite similar, but the striking dif-
ferences in the fruit bodies in these two genera would serve easily to
distinguish between them.

According to Jackson: "It is perhaps unimportant whether one considers *Herpobasidium* a member of the Auriculariales or a simplified form of the Uredinales. In any case it serves to emphasize the probable close relationship between the two groups." *Platycarpa* occupies an intermediate position between the Septobasidiales and the Auriculariales similar to that of *Herpobasidium* between the latter order and the rusts.

It seems that *Platycarpa* occupies a rather significant position in the evolution of such a highly specialized order as the Septobasidiales. In the Uredinales the most primitive genera grow on ferns. In the Septobasidiales no species has yet been found growing on ferns, but it is probable that *Platycarpa* might represent a sort of ancestral type of this order. At any rate, *Platycarpa,* which so strikingly resembles *Septobasidium* and is excluded from that genus only because of the fact that it is not associated with scale insects, furnishes a good connection between the Auriculariales and the Septobasidiales.

Uredinella, parasitic on scale insects, represents a similar transitional form between the Uredinales and the Septobasidiales, being close to *Septobasidium* in its association with scale insects and related to the rusts in that the "hymenial" layer in one species may be composed of a compact layer of teleutospores without sterile threads (Couch, 1937) and thus is a true sorus. Indeed the teleutospores of *Uredinella coccidiophaga* are so much like those of certain species of *Uromyces* (*e.g., U. Fabae*) that it is difficult to know whether *Uredinella* should be in the Uredinales or in the Septobasidiales. Thus we find rather striking interconnections between these three orders: *Jola* and *Platycarpa* connect the Auriculariales and the Septobasidiales; *Herpobasidium, Goplana,* and other genera represent intermediates between the Auriculariales and the Uredinales; while *Uredinella* is intermediate between the Septobasidiales and the Uredinales. In spite of the fact that these orders are so obviously interconnected it would seem unwise to merge them into one or two at present. The Uredinales comprise a highly specialized group of obligate parasites which have for the most part a rather poorly developed vegetative mycelium but through "chemical stimulators" have put their hosts to work for them in the accumulation

of food for reproductive purposes.   The Septobasidiales represent a parallel and very highly specialized order living symbiotically with colonies of scale insects and parasitizing certain individuals. The vegetative or nourishing mycelium in this order is rather poorly developed but is exceedingly highly specialized, absorbing food material from the living parasitized insects.   The Auriculariales, containing the Auriculariaceae and the Phleogenaceae, are made up of a hodgepodge of genera.   Some of these are interrelated while others may not even belong in the Heterobasidiomycetes.   All of these genera with the possible exception of *Herpobasidium* have a well-developed vegetative mycelium.   The commonest, best known, and most typical genera in this order (*Auricularia, Helicogloea,* and *Platygloea*) have well-developed fruit bodies and are saprophytic except for one or two species which are facultative parasites.   (The parasitic genera in this order are discussed by Fitzpatrick (1918).)   Though no one has made a study of the vegetative mycelium in any of these saprophytic genera, it is very likely that in all of them the nourishing mycelium is quite extensive. In addition to the rather striking physiological distinctions between these three orders, which I have emphasized above, there are also morphological differences.

Helicogloea alba (Burt) n. comb.

Septobasidium album Burt (**1926**)

Resupinate, forming small, circular, white, firm patches 1–6 sq. cm. in area; margin sharply determinate; surface resembling kid, pulverulent under a lens, broken here and there by irregular cracks. In section 600–700 μ thick, whitish throughout, composed of two rather indistinctly differentiated regions, a lower and an upper, each of about equal thickness.   The lower region composed of diagonally ascending, entangled, fairly compact hyphae which are about 4–5 μ thick, have rather thick walls and are furnished with conspicuous clamp connections at the septa.   The upper part of the fungus is composed of hyphae which are much coiled, are very compactly arranged, and 2.8–3 μ thick.   Hyphae throughout both regions rather sparingly septate and usually with clamp connections at each septum; at the upper surface of the fungus the ends of the hyphae spirally coiled.   Probasidia arising from near the bases of these coiled hyphae, usually growing backward or diagonally away
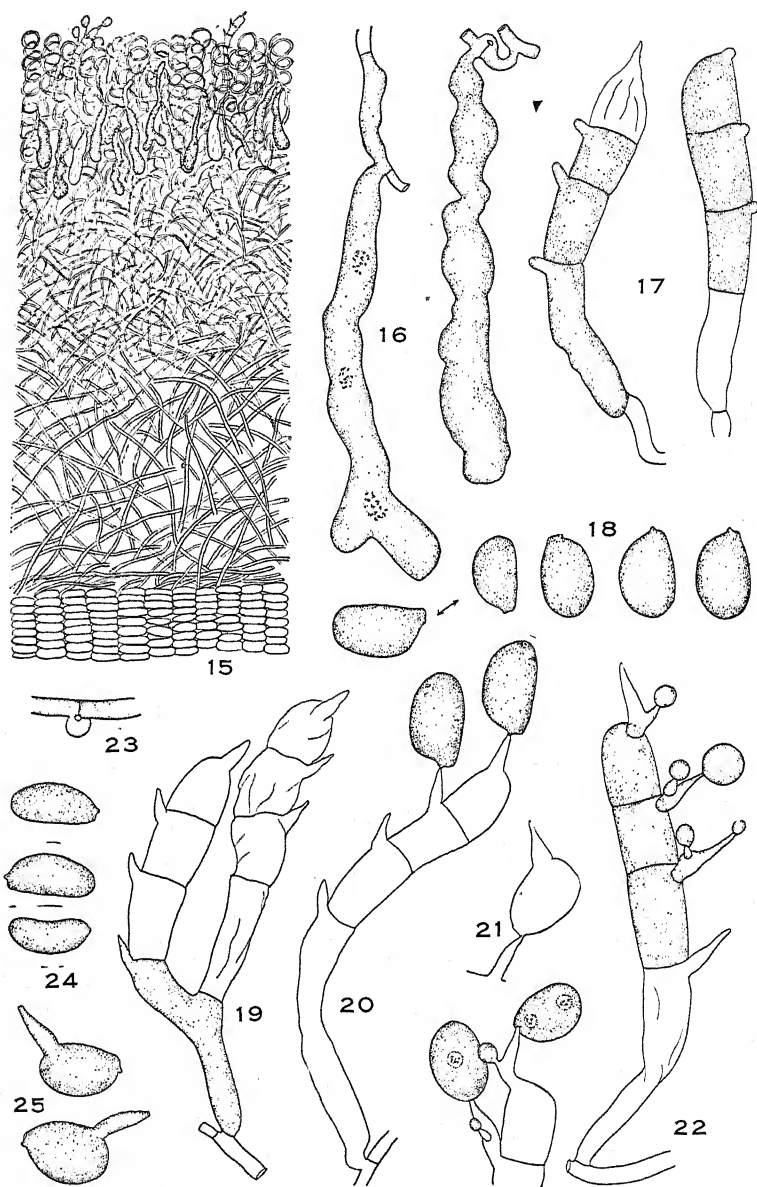
FIGS. 15–23. *Helicogloea alba.*

from the surface of the fungus; formed in clusters on hyphae rich in protoplasm, $7-12 \times 50-70\,\mu$, elongated, club-shaped, thickest at the distal end, constricted at one or several places, straight or bent, sometimes forked, forming a discontinuous layer one to several hundred microns below the upper surface. Rarely the probasidia may grow toward the surface of the fungus. Basidia $8-10 \times 37-50\,\mu$, typically 3-celled, with a long stalk that often forms a sterigma and spore; basidia arising from the base of the probasidium or from the upper part of the·"primordial cell" or rarely from the apex of the probasidium when it is directed upward in the hymenium; sterigmata arising from near the top of each cell, usually short, $5-8\,\mu$ long, sometimes forked into a long and a short branch, bearing respectively a large and a small spore; rarely two sterigmata of equal length may arise on the end of the apical cell of the basidium; spores hyaline, ovoid, $7-9 \times 10-15\,\mu$, slightly flattened on one side and with a truncated proximal end and a minute papilla to which the sterigma is attached; germinating by repetition.

This fungus was first described by Burt as a species of *Septobasidium* but was excluded from that genus by me (1938) because of the close association of *Septobasidium* with scale insects and its invariable habitat on living plants. The position of the present fungus has remained in doubt until this past fall when in the course of studies on a new and remarkable heterobasidiomycete I examined herbarium material of *Helicogloea* and found that the spores of *Septobasidium album* were like those of *Helicogloea*. The spores of this genus, as noted by Baker (1946), are very characteristic. They are typically ovoid to ellipsoid and usually flattened on one side and have a prominent blunt protuberance at the proximal end. The small papillate mucro which is attached to the sterigma is at the edge of the truncation away from the flattened side of the spore (FIGS. 18, 24, 25). Further studies, particularly of sectioned material, showed the much-elongated probasidial sacs from which the basidia arose. Great difficulties were encountered in interpreting the probasidial structures. This was due to the fact that the first material studied was teased apart instead of sectioned and I mistook the irregular probasidia for basidia. It was only after a study of sectioned material which showed the large probasidia with their broader ends directed away from the surface that the relationship between the two structures was partly understood. It was noted further that the probasidia are very irregular in shape, whereas

the basidia are always nearly straight-cylindrical. Material teased apart in 7 per cent KOH, washed and then lightly stained with cotton blue or weak gentian violet and studied immediately, helped in showing a connection between probasidial cells and basidia. The probasidia collapse after they become empty and the usually long and twisted hypha or stalk between the probasidium and the basidium empties into the latter; hence this connection is difficult to follow. Several examples were found in teased material where the empty and partly collapsed probasidium was connected with a partly collapsed basidium. Typical probasidia are shown in figure 16. These are shown hanging downward. They are really growing away from the surface of the hymenium and are pendulous only if the fungus is growing on the upper surface of the substratum, which I strongly suspect it seldom does, and hence the probasidia would usually have the growing (larger) end directed upward (see Boedijn, 1937). In figure 15 the probasidia are shown with their growing points directed away from the surface. This sketch was made from a comparatively thin and young section. In sections of older plants the probasidia are sunken deeper in the tissue and the region of coiled hyphae is considerably thicker. On the far left of the same figure is shown a probasidium germinating at its base to form a basidium initial which is growing toward the surface. The third (from the left) probasidium is empty and partly collapsed and is indistinctly connected by dotted lines to an empty and collapsed basidium. Left of this collapsed basidium is one with spores attached which apparently arose from a probasidium with its growing end directed toward the surface. Third from the far right is a basidium full of protoplasm, connected indistinctly to an empty probasidium.

The probasidia in this genus are furnished with walls so thin that the wall would perhaps serve little or no protective purpose against desiccation. The lack of a thick protective wall is compensated for by the sinking of the probasidia into the tissue. This reaches an extreme in *H. indica* where the probasidia are located near the base of the fructification (Boedijn, 1937).

Baker (1946) points out that the species of this genus fall into two groups, one with a mucous-gelatinous fructification, the other floccose or hypochnoid. The present species is closer to the floc-

cose type but is much more like a *Corticium,* resembling *C. portentosum,* as mentioned by Burt.

Helicogloea alba agrees rather closely with *H. contorta* Baker in the size and color of the fructification, in the structure of the hyphae of the context, particularly in the spirally coiled hyphae near the outer surface, and in the size and shape of the probasidia, basidia, and spores. The two seem to differ in texture, *H. contorta* being floccose while *H. alba* is *Corticium*-like and rather firm and tough.

Specimens examined: New Zealand: Queenstown, Otago. On fallen branches of *Nothofagus,* December 3, 1919. G. H. Cunningham, coll. No. 542, **type.** Part of type in Mo. Bot. Gard. Herb.; U. S. D. A. Herb.; and U. N. C. Herb.

SUMMARY

A new genus of the Auriculariales is described. It is named *Platycarpa* because of its flattened fruit body and is based on two species, both of which are parasitic on tropical ferns. One of these, *Platycarpa boliviensis,* is described here for the first time; *P. Polypodii,* previously described as a *Septobasidium,* is transferred to *Platycarpa* and is considered the type species of this new genus. *Platycarpa* is compared with *Jola* and *Eocronartium* on mosses and *Herpobasidium* on ferns and shown to be distinct from each of these. The possibility that *Septobasidium* may have arisen from such a genus as *Platycarpa* is discussed.

The fungus described by Burt as *Septobasidium album* is transferred to the genus *Helicogloea* as *H. alba* because of the striking similarity in probasidial, basidial, and spore characters, as well as in the vegetative characters.

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EXPLANATION OF FIGURES

Fig. 2. Diagrammatic section of fertile leaflet of *Polypodium* sp. showing
section of fruit body of fungus, *Platycarpa Polypodii,* attached to sori. Hy-
menial region bearing probasidia, basidia, and spores, is on lower surface of
fungus. × 22. Fig. 3. Two prosporangial cells of *Polypodium* sp. showing
coiled haustoria of *P. Polypodii.* Disorganized contents of host cells not
shown. × 543. Fig. 4. Section of hymenial layer of *Platycarpa Poly-
podii* showing sterile hyphae, probasidia, basidia, and spore. × 580. Figs.
5, 6. Basidium and three spores of *P. Polypodii.* × 543. Fig. 7. Dia-
grammatic section of *Platycarpa boliviensis* showing part of fern leaf,
context and probasidia (p.b.). Section made after soaking in 7 per cent
KOH. × 22. Fig. 8. Section of hymenial region of *P. boliviensis* showing
proliferating probasidia and curved threads of hymenium. × 500. Figs. 9–
13. Basidia and spores of *P. boliviensis.* × 500. Fig. 14. Part of section
of lower epidermis and mesophyll cells of fern leaf showing nourishing
hyphae of *P. boliviensis* within cells. Leaf first boiled in 7 per cent KOH
for ten minutes before cutting section. × 860.

Figs. 15–23, *Helicogloea alba;* fig. 24, *H. caroliniana;* fig. 25, *H. pinicola.*
Fig. 15, × 192; fig. 23, × about 600; others, × 675. Fig. 15. Cross section
of young fructification of *Helicogloea alba,* semi-diagrammatic. For explana-

tion see text. Fig. 16. Two probasidia. Fig. 17. Two basidia. Fig. 18. Five spores. Fig. 19. Two basidia attached to same branched probasidium(?). Fig. 20. Basidium with long stalk-cell. Fig. 21. Spore germinated by repetition attached to sterigma of basidium. Fig. 22. Basidia with branched sterigmata. Fig. 23. Clamp connection. Fig. 24. Spores of *H. caroliniana,* one of which is slightly bent-ellipsoid; others flattened on one side and showing truncated end with a little mucro. Fig. 25. Two spores of *H. pinicola* (Galzin No. 20555) germinating by repetition, both showing truncated proximal ends with small papillate mucros.

OXYPORUS NOBILISSIMUS AND THE GENUS OXYPORUS IN NORTH AMERICA

WM. BRIDGE COOKE

(WITH 10 FIGURES)

Attention has been called to the existence of this genus of pore fungi in North America by the discovery in 1943, and the subsequent collection between 1943 and 1948 at several locations in Oregon and Washington, of specimens of a most unusual species of *"Fomes."*

In July, 1948, the writer visited the Botany Department at the University of Washington in Seattle and was shown and lent a portion of a large fungus which had been given to D. E. Stuntz. Several specimens collected by A. H. Smith and D. E. Stuntz (FIGS. 8–10) in Mount Rainier National Park intensified the writer's desire to know more about the specimen obtained in Seattle. A lead was available through Charles Gardner Shaw of the Department of Plant Pathology, Washington State College of Pullman, Washington. Through conversations, and later correspondence, with C. R. Allison, he learned of a giant *"Fomes"* collected in Lewis Co., Wash., which had been placed on display in the office of the Weyerhauser Lumber Co., Longview, Wash. It was discovered that other similar but smaller conks had been sent to L. O. Overholts by J. L. Bedwell of the Portland Office of the Division of Forest Pathology, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Dept. of Agriculture. It became referred to facetiously by foresters who were acquainted with it as *"Fomes fuzzii-sandozii"* because of its fuzzy appearance and the fact that the first specimens found in Clackamas Co., Ore., in 1943, were collected by the brothers Ali and Fred Sandoz. In 1946, Jacob Hisey found a large specimen, one of the largest fungus fruit bodies known, in Lewis Co., Wash. It weighed at least 300 lbs. and measured 56×37 inches. At least, among

the polypores, this size may be equaled only by some exceptional conks of *Fomes officinalis*.

The following description of the genus *Oxyporus* was translated from Donk's (8) treatment of the Aphyllophoraceae of The Netherlands:

Oxyporus (Bourd. & Galz.) Donk

Oxyporus (Bourd. & Galz.) Donk, Rev. II. Med. Bot. Mus. Utrecht, No. 9, p. 202. 1933.
Coriolus Sect. IV. *Oxyporus* Bourd. & Galz. Soc. Myc. Fr. Bul. 41: 139. 1925.
Polyporus Fr., Syst. Myc. 1821. pr.p.

Fructification stalked, resupinate-reflexed or wholly resupinate, perennial. Pileus, when present, anoderm. Tubes at least two- to many-layered, rarely remaining one-layered; pores small, oval-rounded. Trama woolly-corky, somewhat fibrous, white or at most pale leather colored, occasionally strongly reduced.
Cystidia present, with a cap of calcium oxalate crystals on the apex. Spores ovate-rounded, smooth, hyaline, small (3.5–5 μ).
Upon wood.
Type: *Polyporus populinus* Fr.
All treatments of this genus studied to date list only one principal species, *Fomes connatus* or *F. populinus*, with a secondary species, *Poria obducens*, which is sometimes listed as a synonym, a sub-species, or a variety of *Fomes connatus*. Bondarzew and Singer (2) indicate the inclusion of *O. ravidus*, a non-stratose species not reported from North America, treated as a *Trametes* by Pilát (19).

Within the genus *Fomes* Kickx (10) there are several groups of species which seem to have little relationship to each other. Fries (9), followed later by Winter, Killermann and others, divided the genus into six or more sections, related to each other only by the presence of a tubular hymenium, perennial habit, and woody or near-woody structure. In the section "Laevigati" were placed species which were not rimose, did not have a crust, but whose surface was smooth. Into this section, then, fell the fungus referred to as *Fomes populinus* or *F. connatus*.

In splitting *Fomes*, Karsten erected *Fomitopsis* for species with white or light colored context and in this he is followed at present, to a certain extent, by Cunningham (7).

Murrill (15) retained *Fomes* for the light-context species. He used other generic categories for perennial *Fomes* with dark context and other characters.

Other workers in the United States including Overholts (18), Lowe (14), Baxter (1), Shope (20), Neuman (16) and others have used the genus *Fomes* in the sense in which it was used by Fries.

In 1925, Bourdot & Galzin (3) in France established a subgenus in the genus *Coriolus* for *Fomes connatus*. Quélet had already placed this species in that genus and Bourdot and Galzin set it apart from other species as the subgenus *Oxyporus*. In 1933, M. A. Donk (8) elevated *Oxyporus* to generic rank. He placed two common species in this genus: *Fomes connatus* and *Poria obducens*. Pilát (19) in Czechoslovakia, Imazeki (11) in Japan, Bondarzew and Singer (2) in Russia, and Singer in the revised Farlow Herbarium arrangement, have followed Donk in this although Bondarzew and Singer have indicated that they have emended the genus to include an acystidiate form of *O. obducens* and a non-stratose species, *O. ravidus*. Included with *Oxyporus* in the tribe *Oxyporeae,* Bondarzew and Singer place the genera *Baeostratoporus, Heteroporus* and *Irpex.*

KEY TO SPECIES

a. Pileus with a deep surface layer of interwoven fibers; on conifers; spores 6–7 × 3.5–4 µ ....*O. nobilissimus*
a. Pileus with smooth to pubescent surface; on hardwoods; spores 3.5–4.5 × 3–4 µ ....*O. populinus*

Oxyporus nobilissimus sp. nov. FIGS. 1–3, 8–10

Pilei dense strigosi, fibris ramosis hispidi, zonati, suberoso-lignosi, effuso-reflexi vel substipitati, sicutae imbricati, concrescentes, perenne, 30–140 × 25–95 × 30–100 cm., albi cinereive, intus poris stratosis, carne mediocri, 2–5 mm. crassa, dense fibrosa; margine obtuso, sterili; tubulis 2–7 mm. longis, ore rotundatis, in juventute albis, 400–500 µ diam.; hyphis longis, hyalinis, raro ramosis, non septato-nodosis; cystidiis hyalinis, capitatis, crystallinis, 15–23 × 3–6 µ; basidiis tetrasterigmaticis, 12–15 × 4–5 µ; sporis ellipsoideis, hyalinis, uniguttulatis, unicellularibus, 6–7 × 3.5–4 µ.

Hab. ad truncos *Tsugae heterophyllae,* Mt. Rainier National Park, Washington. Leg. A. H. Smith et D. E. Stuntz, A. H. Smith 31102. Sept. 9, 1948.

Pileus perennial, sessile, or centrally substipitate, ungulate, imbricate, massive, 30–140 × 25–95 × 30–100 cm., at least one speci-

men weighing as much as 300 lbs., distinctly stratified from each
season's growth, with a thin layer of sterile tissue between succes-
sive trametoid tube layers. Color of old context more or less cin-
namon-buff or more ochraceous, fresh growth white to sordid white
and fibrous, texture when fresh semi-woody; surface of pileus a
dense mat of white mycelial fibers composed of bundles of parallel,
elongate, septate, thin- to slightly thick-walled hyphae; the bundles
branch and anastomose freely and the fiber mat shows evidence of
seasonally added increments of growth.
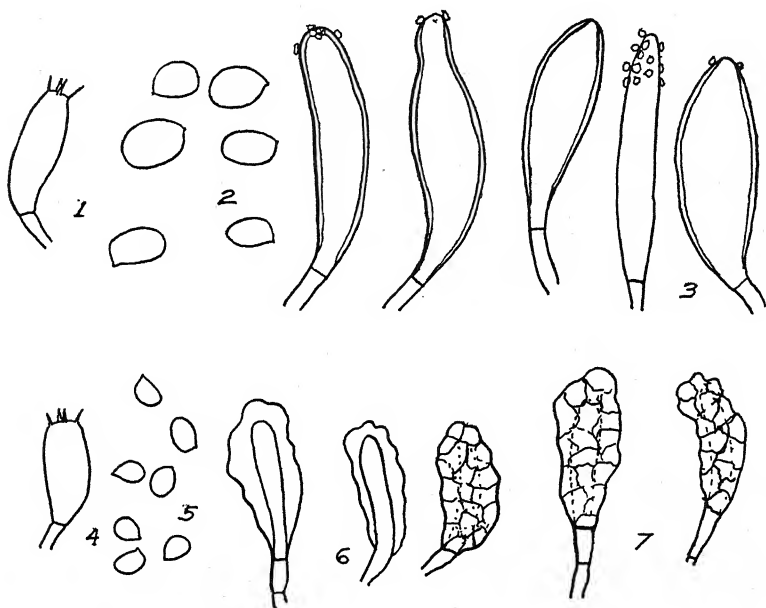
FIGS. 1–7. *Oxyporus nobilissimus* and *O. populinus.*

Hyphae of context flexuous, hyaline, 3–5 μ in diameter, walls
thin to slightly thickened, of two types: generative hyphae terminat-
ing in the hymenium, thin-walled, flexuous, septate but not with
clamp connections, intermingled with the thin- to slightly thick-
walled interwoven skeletal hyphae which emerge from the upper
surface of the pileus to form, in parallel arrangement, the mat fibers.

Tubes 2 per mm., white, round, 2–3 mm. deep in a new layer on a
fresh specimen collected in September, 2–7 mm. deep in mature
layers, averaging 5 mm. deep, not becoming stuffed; 35 seasons of
growth counted in one of the Mt. Rainier specimens; in all speci-
mens examined the youngest tube layer white and the tube trama
trametoid in structure.

Cystidia hyaline, walls slightly thickened, apex with a crystalline cap, 15–23 × 3–6 μ. Basidia 4-spored, 12–15 × 4–5 μ. Spores 1-celled, hyaline, smooth, thin-walled, 6–7 × 3.5–4 μ.

Habitat: On *Abies procera* Rehder and *Tsuga heterophylla.*

Distribution: Clackamas Co., Oregon; Lewis Co. and Mt. Rainier National Park, Washington.

Type specimen: Collected by A. H. Smith and D. E. Stuntz, A. H. Smith No. 31102, on *Tsuga heterophylla,* Mt. Rainier National Park, Washington, Sept. 9, 1948. Portions of this specimen are filed in the Herbarium of the University of Michigan; the Mt. Rainier National Park Herbarium, the herbarium of the Univ. of Wash., Seattle, Wash., the herbarium of Wm. B. Cooke, and that of D. V. Baxter.

Important specimens: Collected by Jacob Hisey, July 13, 1946, on *Abies procera* at location 559, N.E. ¼ of S.E. ¼, Sec. 5, T. 11 N., R. 4 E., Lewis Co., Washington. Portions of this specimen are in the Herbarium, Dept. of Plant Pathology, State College of Washington, Pullman; Herbarium, University of Washington, Seattle; and the writer's herbarium. The main part of the specimen is on display in the office of the Weyerhauser Lumber Co., Longview, Wash.

To the writer's knowledge, the first specimens of this species were collected by Ali and Fred Sandoz on *Abies procera* on the Dwyer Brothers operation on the Mt. Hood National Forest in the Clackamas Drainage near Oregon City, Clackamas Co., Oregon, June 10 and July 10, 1943. These specimens were turned over to the Portland office of the Division of Forest Pathology where specimens are now filed and from whence specimens were sent to Dr. L. O. Overholts in whose herbarium at Pennsylvania State College two specimens are filed under the numbers 23909 and 24218. A portion of one specimen was also sent to the Forest Pathology Collections at Beltsville, Maryland.

These first specimens were found associated with living trees of *Abies procera* within 5–6 feet of their base, according to the Allison correspondence, within 3 feet of their base according to correspondence with J. L. Bedwell. Attempts to trace the fructifications to the nearby trees were unsuccessful and incomplete; felled trees were reported by Allison, in correspondence, to have shown no signs of infection. The specimens from Mt. Rainier seen by
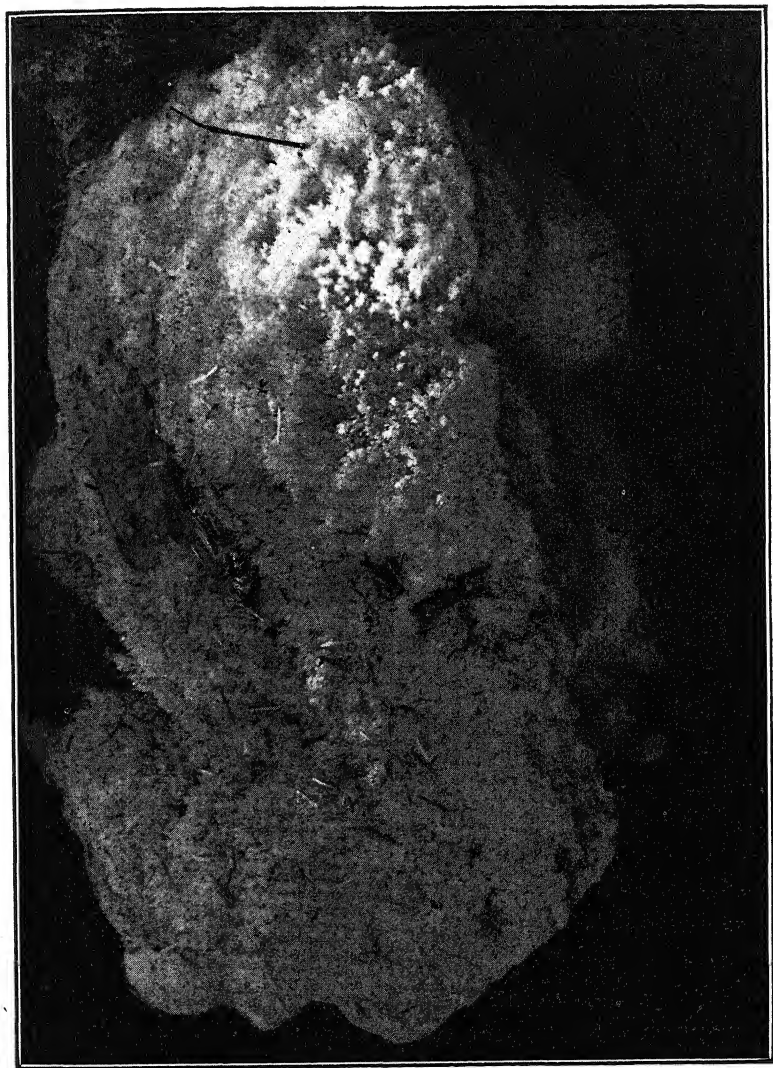
Photo A. H. Smith.

Fig. 8. *Oxyporus nobilissimus.* × ⅛.

the writer are associated with a brown rot of lowland hemlock (*Tsuga heterophylla*).

C. G. Shaw at the Dept. of Plant Pathology, State College of Washington, attempted to make tissue cultures from one of the specimens collected on Mt. Rainier. The specimen was collected in September and the cultures were attempted without success in November.

Aside from the large size of most of the known conks of this fungus it has many interesting features which set it aside as distinct from all other known species of the genus *Fomes* and its segregates except *Oxyporus populinus* described below. Upon first glance one is struck by its similarity to this fungus, which grows only upon hardwood trees. The tubes are separated by layers of white tissue and the context is white (FIG. 10), but there the macroscopic similarity ends. Microscopically, both species have capitate incrusted cystidia in the hymenium (FIGS. 3, 6, 7).

The surface of the specimens of *O. nobilissimus* is covered with loosely interwoven, stiff, matted fibers giving the appearance (FIGS. 9–10), as suggested by A. H. Smith in his field notes, of some *Tremellodendron* species. The individual fibers of this mat are rounded to flattened. They are composed of elongate, hyaline, thin- to slightly thick-walled septate hyphae which are arranged parallel or somewhat interwoven but densely compacted. These hyphae do not have clamps, are not flexuous and are not gelatinous. The fibers which are made up of these long straight hyphae are 50–200 μ in diameter, and branch and anastomose freely. The mass of fibers originates from the context in which the hyphae which produce the fibers are densely interwoven with other hyphae. The fibers leave the context, with the hyphae which compose them, already in their parallel arrangement. These bundles of hyphae leave the context parallel to it, tangential to the cap surface, and are renewed at intervals (FIG. 10). These intervals may not correspond to the seasons of growth in which the tubes are renewed in additional layers but their effect is similar. Thus within this surface layer, which may be as much as 5 cm. deep, there may be a number of increments of this tissue distinguishable by white to light colored areas probably indicating areas of renewed growth in-
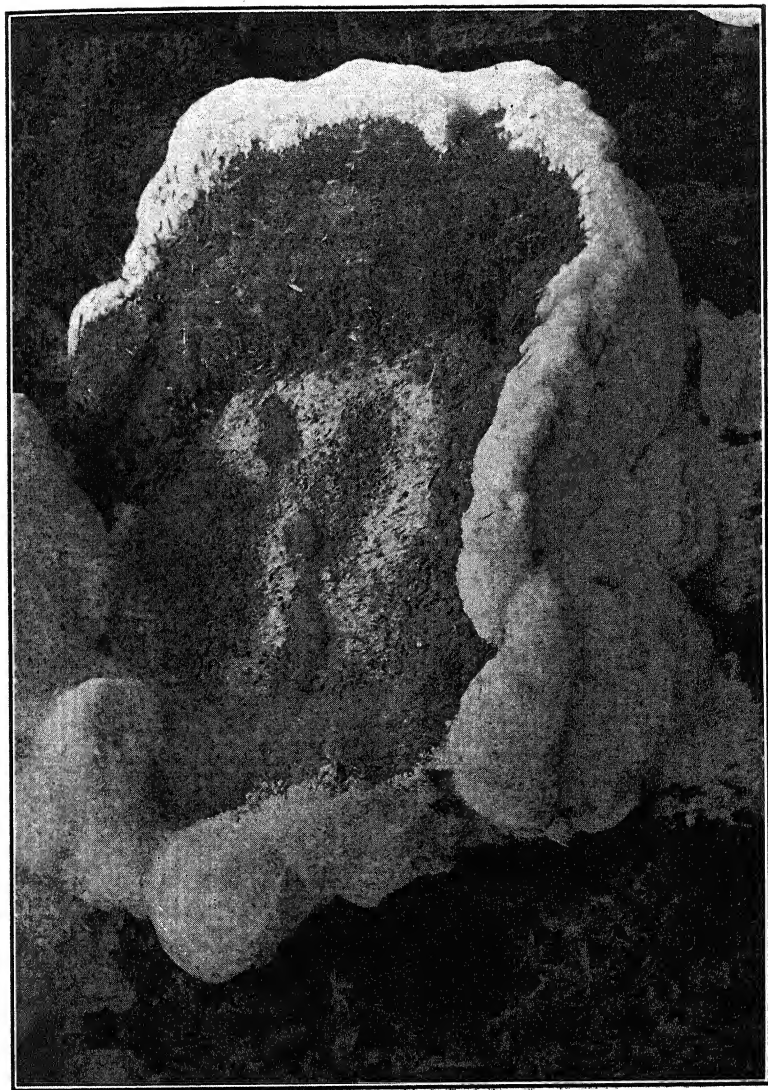
Photo A. H. Smith.

Fig. 9. *Oxyporus nobilissimus* (36 inches wide).

tensified at the start of a growing season. Where the many fibers appear perpendicular to the conk-surface (anticlinal rather than periclinal) this is probably caused by crowding through increasing growth, or realignment in older areas.

The context of the sterile tissue between the tube layers and above the oldest tube layer beneath the fiber mat is composed of densely interwoven hyphae which are hyaline, septate, not clamped, 3–4 μ in diameter. The context is trametoid, there is no distinction between the tube trama and the context. The sterile tissue between the tube layers is white and varies in thickness from 0.25 to 3.0 mm., but is mostly between 0.5 and 2 mm. (FIG. 10). This tissue is leathery to corky or woody. Between the tube layers and the fiber mat at the upper surface, the context is between 0.5 and 2 mm. thick.

In treating certain tropical polypores E. J. H. Corner (4, 5) developed a system of classification of tissues within the trama on the basis of the types of hyphae of which they were composed. This system was recently further elaborated and used as the basis for the systematic treatment of New Zealand polypores by G. H. Cunningham (6). According to this system of hyphal classification two hyphal types are represented in the Mt. Rainier specimens as well as in the Lewis Co. giant. The generative hyphae are those which produce the basidia and the cystidia. The greater part of the tissue of the conk is composed of skeletal hyphae. These hyphae are of Cunningham's "long type" and they are the hyphae which develop into the elongate fibers of the surface mat. Binding hyphae are not present so that this fungus represents a dimitic hyphal type without clamp connections. In Cunningham's classification (7) the fometoid fruit body with a dimitic hyphal system without clamp connections is characteristic of *Fomitopsis*. This concept represents an emendation of Karsten's original concept of this genus.

In the section devoted to cover tissues in Lohwag's "Anatomie" (13) there is no mention of a tissue such as the one on the surface of the conks of this fungus. Lohwag's cover tissues are all relatively simple ones and the hyphae of the different kinds of derms and cutis are relatively simple, not compound bodies such as the fibers of the mat described above. Study of other compound
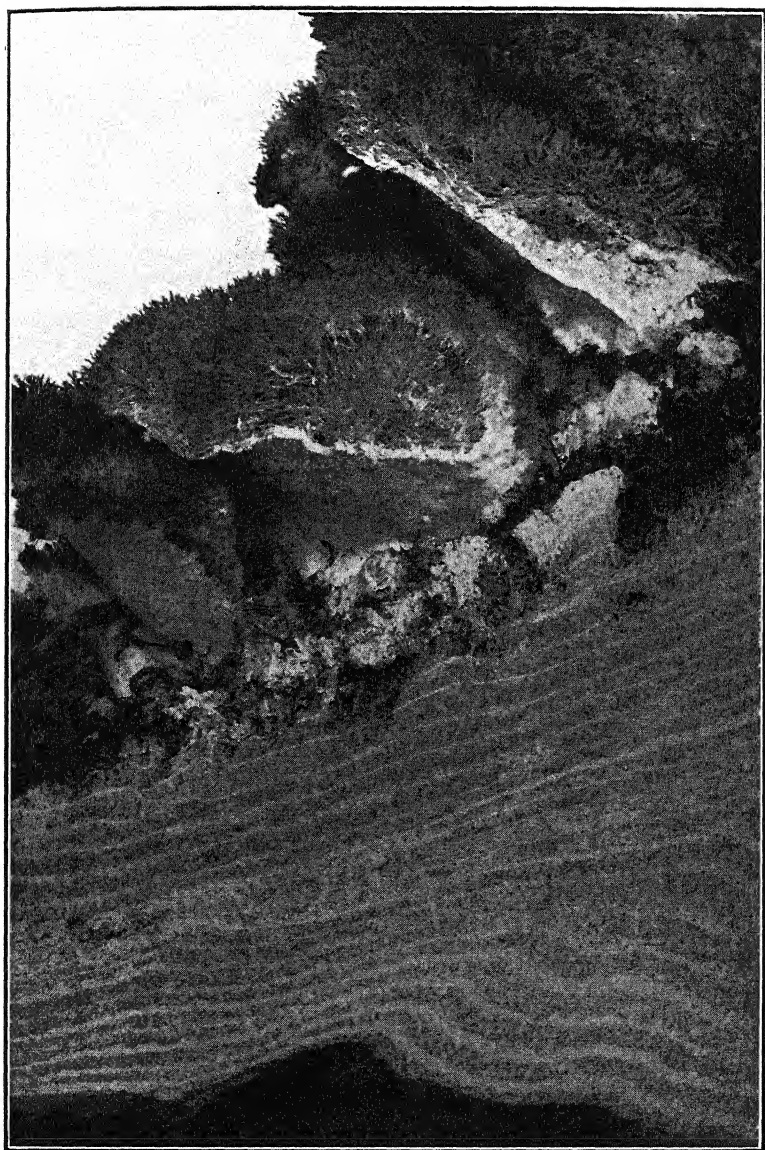
Photo A. H. Smith.

Fig. 10. *Oxyporus nobilissimus.*   × ½.

trichoderms such as those of *Pogonomyces hydnoides, Funalia mons-veneris* and *Trametes hispidus* would be necessary in order to properly evaluate the cover tissue of this fungus.

Until recently *Oxyporus populinus* was the only fungus found in North America which could be assigned to this genus. No other species which had layers of white context between the layers of tubes was known to Lloyd in 1915 (12). Baxter (1), in 1948, says "there are no closely allied species." Overholts said, in part, in a letter to J. L. Bedwell dated June 30, 1943: "It appears to be a good species of *Fomes* of the white context group but the pileus covering is so different from any other *Fomes* that I am at a loss as to what to do with it. I have had nothing like it previously."

OXYPORUS POPULINUS (Fr.) Donk

Boletus populinus Schum., Sael. p. 384. 1803—"varietas videtur"—Fr.
Polyporus populinus Fr., Syst. Myc. **1**: 367. 1821.
Polyporus obducens Pers., Myc. Eur. **2**: 104. 1825.
Polyporus neesii var. *connatus* Weinm. ex Fr., El. **1**: 92. 1828.
Polyporus connatus (Fr.) Weinm., Fl. Ross. 332. 1836—non *Polyporus connatus* Schw., Am. Phil. Soc. Trans. ii. **4**: 154. 1832.
Fomes connatus Gill., Champ. Fr. **1**: 684. 1878.
Polyporus oxyporus Sauter, Hedw. **15**: 150. 1879.
Fomes populinus (Fr.) Cke., Grev. **14**: 20. 1885.
Fomitopsis connata Karst., Rev. Myc. **3**: 18. 1881.
Trametes connata Karst., Act. Fenn. **3**: 30. 1881.
Leptoporus connatus Quél., Ench. Fung. 177. 1886.
Poria obducens (Pers.) Quél., Ench. Fung. 180. 1886.
Coriolus connatus (Fr.) Quél., Fl. Myc. 391. 1888.
Physisporinus obducens (Pers.) Gillot & Lucand, Cat. Ch. Saone-et-Loire, 697. 1891.
Polyporus meliae Underw., Torr. Bot. Cl. Bul. **24**: 85. 1897.
Fomes meliae (Underw.) Murr., Torr. Bot. Cl. Bul. **30**: 232. 1903.
Fomes oxyporus Lloyd, Syn. Gen. Fomes, Myc. Writ. **4**: 283. 1915.
Polyporus cremeus Bres. ex Lloyd teste Bres., Ann. Myc. **18**: 67. 1920.
Polyporus ulmarius Velen., C.H. 661. 1922. (non Fr.)
Coriolus connatus ssp. *obducens* (Pers.) Bourd. & Galz., Soc. Myc. Fr. Bul. **41**: 137. 1925.
Oxyporus populinus Donk, Med. Bot. Mus. Utrecht **9**: 204. 1933.
Oxyporus obducens (Pers.) Donk, Med. Bot. Mus. Utrecht **9**: 202. 1933.
Oxyporus populinus var. *obducens* (Pers.) Pilát, Atlas des Champignons de l'Europe, III: 343. 1936-42.

Pileus perennial, sessile to resupinate, convex, imbricate, corky to woody, 2–10 × 3–15 × 0.5–4 cm.; surface white to yellowish,

sometimes becoming grayish black, often becoming covered with moss and algal growth, velvety tomentose to glabrous, not zonate, never rimose; margin thick, usually narrowly sterile below; context white or whitish, drying white to cream color, soft, spongy to nearly succulent when fresh, corky when dry, 0.3–4 cm., usually less than 2 cm., thick, the hyphae rarely branched, thin-walled, 2–5 μ in diameter, generative and skeletal hyphae densely interwoven; very similar to each other; tubes white or whitish, distinctly stratified, the layers of tubes separated from each other by a layer of context 0.5–1 mm. thick, 1–5 mm., mostly about 3 mm. long each season, mouths white to whitish or yellowish, often glistening, angular, averaging 4–5 per mm., the edges entire to slightly dentate, rather thin.

Cystidia present although not reported from all specimens, capitate or club-shaped, 11–19 × 3–7–11 μ in diameter, mostly appearing as crystalline bodies in the hymenium; basidia 7–9 × 4–5 μ; spores hyaline, smooth, sometimes 1-guttulate, ellipsoid to globose, 3.5–4.5 × 3–4 μ.

Habitat: Baxter and others list the following woody species as host to this fungus: *Acer dasycarpum* var. *lutescens, A. macrophyllum, A. negundo, A. rubrum, A. saccharinum, A. saccharophorum, Aesculus hippocastanum, A. octandra, Betula lutea, Carpinus caroliniana, Carya glabra, Carya* sp., *Cornus florida, Fagus grandifolia, Fraxinus americana, Gleditsia triacanthos, Halesia carolina, Liquidambar styraciflua, Melia azederach, Nyssa silvatica, Ostrya virginica, Quercus alba, Q. prinus, Sambucus* sp., *Ulmus americana* and *U. fulva.*

Within North America the following provinces and states are listed as areas within which this fungus has been collected: Canada: British Columbia, Manitoba, New Brunswick, Nova Scotia, Ontario, Prince Edward Island and Quebec. United States: Alabama, Arkansas, Delaware, Illinois, Indiana, Kansas, Kentucky, Iowa, Maine, Maryland, Massachusetts, Michigan, Minnesota, Missouri, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Pennsylvania, Rhode Island, South Dakota, Tennessee, Vermont, Virginia, West Virginia and Wisconsin. Pilát reports it from the following European countries: Czechoslovakia, Hungary, Germany, France, Norway, Sweden, Esthonia, Finland, Poland, The Netherlands, Jugoslavia, Latvia and Russia. M. C. Cooke reported it from Australia, according to Pilát.

Its pathology and activity in pure culture are adequately described by Baxter (1) and Nobles (17).

In its resupinate form it has been referred to as *Poria obducens* Pers. Workers in North America have usually considered this species as synonymous with *O. populinus* and we shall follow Lowe (14), Baxter (1), and others in this interpretation.

The above description of *O. nobilissimus* was drawn from field notes by A. H. Smith and laboratory notes by the writer. The description of *O. populinus* was drawn from several descriptions including those of Lowe (14), Donk (8), Baxter (1), Pilát (19), Bourdot and Galzin (2), and from notes by the writer. The synonymy of the latter species was drawn from Pilát (19), Donk (8), and Baxter (1). In addition to the above synonyms Pilát lists "*Polystictus populinus* var. *connatus* Fr., El. 92. 1825." This is an inaccurate citation since Fries did not raise *Polystictus* to generic rank until 1851 and the Elenchus was published in 1828. Host and location lists were obtained mostly from Baxter (1).

The writer wishes to take this opportunity to thank Donald P. Rogers for checking the Latin description of *Oxyporus nobilissimus;* A. H. Smith, D. V. Baxter and C. G. Shaw for reading the manuscript and offering valuable criticisms and suggestions; and A. H. Smith for use of his photographs of the Mount Rainier specimens.

DEPT. OF BOTANY,
STATE COLLEGE OF WASHINGTON,
PULLMAN, WASH.

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DESCRIPTION OF FIGURES

Figs. 1–3. *Oxyporus nobilissimus:* 1, basidium; 2, spores; 3, cystidia. Figs. 4–7. *Oxyporus populinus:* 4, basidium; 5, spores; 6, cystidia in optical section; 7, cystidia showing incrustation.

Fig. 8. One year's growth of *Oxyporus nobilissimus* showing the tufted nature of the fibrils forming the covering of the pileus. × ⅛.

Fig. 9. Old fruiting body of *Oxyporus nobilissimus* showing growing margin and matted fibrils forming the covering of the pileus. The fruiting body measured about 36 inches across.

Fig. 10. Sectioned fruiting body of *Oxyporus nobilissimus* showing layer between the tube strata and rejuvenation from pileus surface. × ⅛.

SCHWEINITZ–FRIES LETTERS

C. L. Shear

(with 2 figures)

The following letters represent the only correspondence so far known between these two distinguished mycologists. As may be noted in the transcription of Fries' letter, he lists those decades of his Scler. Suec. sent to Schweinitz at that time, stating that the lacking numbers would follow. A check of the specimens from Fries in the Schweinitz herbarium, as represented in the Michener Collection, showed no specimens among those numbers which were to be sent at a later date. This would indicate that no additional specimens were received by Schweinitz and apparently no further correspondence ensued. No other letters have been found at Philadelphia or Uppsala.

The original Schweinitz' letter is preserved in the Botanical Institute of the University of Uppsala, Uppsala, Sweden. A photostat copy of this was provided through the courtesy of Dr. J. A. Nannfeldt and has been transcribed and translated by Dr. G. Steiner of the United States Plant Industry Station, Beltsville, Maryland. The copy of Fries' reply, which is at the Philadelphia Academy of Natural Sciences, was kindly supplied by Dr. F. W. Pennell of the Academy and translated by Miss Edith K. Cash of the Plant Industry Station. To all the above who have so kindly assisted in making the publication of these letters possible, we wish to express our gratitude.

Bethlehem, Northampton County,
Pennsylvania
17th Jany. 1823.

Hochgeehrter Herr

Die Güte meines Freundes Liliencron, dessen Gesellschaft ich schon über ein Jahr genossen, und der im Begriff ist in sein Vaterland zurückzukehren, verschafft mir die längst gewunschter Gelegenheit, zumal derselbe sich Ihrer persönlichen Bekanntschaft

456

erfreut, Ihnen schriftlich die Hochachtung zu begrüssen, welche
mir Ihre unschatzbaren Arbeiten für mein Lieblings-studien die
Mycologie einflössten, und es zu wagen Ihnen eine kleine Sendung
von etlichen hundert Nord. Americ. Fungi in der Hoffnung zu
übersenden, dass dieselben Ihnen angenehm sein werden. Eine
beträchtlichen Theil derselben werden Sie in dem kleinem Werk
finden, welches Dr. Schwägerichen in der Leipziger Gesellschaften
Schrift von mir hat einrücken lassen. Die übrigen sind seitdem
gefunden, und so viel ich weiss nur in meinen ungedruckten Papieren
beschrieben. Wenn es Ihnen angenehm wäre würde ich im Stande
sein Ihnen von den 1600 Species die ich bisher hier und an meinem
früheren Wohnort in Nord Carolina beobachtet habe (von denen
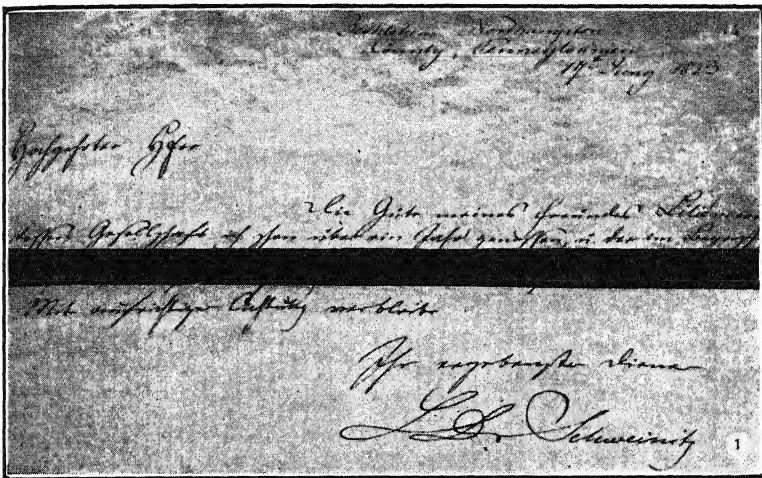
FIG. 1. Heading and conclusion of letter from Schweinitz to Fries.

jedoch noch 1200 auch europ. sind). Exemplare zuschicken, von
den meisten welche preferable sind. Ihr Werk besitze ich bis ende
die Fung. Clavat. und habe es nützlich gefunden. Seitdem ich
mit Dr. Nees v. Esenbeck's und Ihrem System bekannt bin, habe
ich dasselbe ganz angenommen und meine Fung. darauf rangiert.
Wenn mir es die Zeit erlaubte könnte ich Ihnen viel interessante
und für die Physiologie der Schwämme nicht unwichtiges aus
meiner Beobachtung mitteilen, welche ich ein anderes Mal zu thun
mir Erlaubnis erbitte, wenn es Ihnen angenehm ist. Ich habe
Ihrer Sendung ein Verzeichniss das in meiner Sammlung (welche
gegen 3000 Cryptogamisches enthielt) fehlenden Species beigelegt,
nicht ohne Hoffnung dass Sie vielleicht Gelegenheit finden möchten

mir ein und anderes mitzutheilen. Sollten Sie die Gewogenheit
haben mit mir eine correspondz zu wünschen so wollte ich bitten
irgend etwas mir bestimmtes unter der Address Rev. L. D. de
Schweinitz, Bethlehem N. County Pennsylvania durch den Schwedi-
schen Consul in New York an mich gelangen zu lassen. Auch
phaenogamische Seltenheiten wurden mir angenehm sein. Die
Kürze meiner Zeit—da ich eben im Begriff bin mir weiter Reise in
Amtsgeschäften nach Ohio anzutreten—zwingt mich abzubrechen
und Sie nur zu bitten das Sie es mir gütigst verzeihen dass ich
Ihnen vielleicht mit diesen Ansprüchen beschwerlicher falle, Ich
schiebe alle Schuld auf meinen frd. Liliencron, der mich dazu
ermuntert hat—und der Ihnen von meinen hiesigen Verhältnisse
erforderlichen falls Auskunft geben wird. Mit aufrichtiger Ach-
tung verbleibt

<div style="text-align:center">Ihr ergebenster Diener</div>

<div style="text-align:right">L. D. v Schweinitz.</div>

<div style="text-align:right">Bethlehem, Northhampton County,
Pennsylvania.
17th January 1823</div>

My dear Sir:

My friend Liliencron, whose company I have enjoyed for over a
year, is about to return to his homeland. Since he is so fortunate
as to be one of your acquaintances, his kindness made it possible for
me to write this letter, a pleasure I had anticipated for a long time.
Your invaluable papers on mycology, a branch of science which is
also my favorite, have my highest esteem, and I am taking the liberty
of sending you a few hundred fungi of North America, hoping that
this will please you. A considerable part of these fungi you will
find mentioned in my little contribution which Dr. Schwägerichen
had submitted for publication to the Leipzig Society. The others
have been found since and as far as I know are only described in my
unpublished papers. If agreeable to you, I could send the most
desired of the 1600 species which I have found here and in my
previous dwelling place in North Carolina. (However 1200 of
these are also found in Europe.) I have your book to the end of
the Fungi Clavat. and found it useful. After becoming familiar
with the system of Dr. Nees von Esenbeck. a system which is also
yours, I have accepted it fully and have classified my fungi accord-
ingly. If time would permit, I could inform you of many interest-
ing facts observed by me, and which are by no means unimportant
for the physiology of the fungi. With your permission I shall do
so at some other time. My shipment to you contains a list of all
species which are missing in my collection (which has nearly 3000

Cryptogamicae), hoping that you might have time to let me have this or that. If you should be so kind as to wish to enter into correspondence with me, I would ask you to send anything designated to me under the address of Rev. L. D. de Schweinitz, Bethlehem, Northhampton County, Pennsylvania, care of the Swedish consul in New York. It would please me to receive also phanerogamic rareties. Lack of time—I am about ready to leave for a long official trip to Ohio—forces me to cut this letter short. Please pardon me kindly if my request should inconvenience you. I am putting the blame entirely on my friend Liliencron, who encouraged me to write you, and who will be able to give you all the information about the conditions under which I am living here.

With sincere respect I remain

Your most obedient servant,

L. D. v. Schweinitz
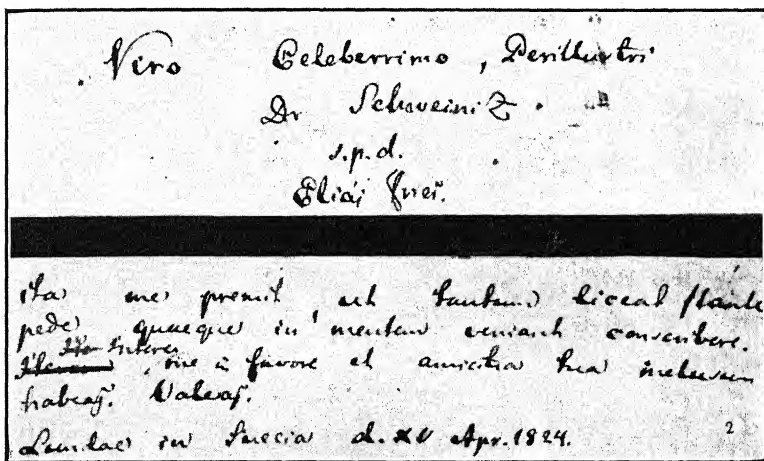
FIG. 2. Salutation and conclusion of Fries' reply to Schweinitz.

Viro celeberrimo, perillustri Dr. Schweinitz s. p. d. Elias Fries

Litteras suas humanissimas plantarumque collectionem ditissimam, cum amicissimo Liljecrona missam summa cum voluptate accepi, et quas gratias ago quantas possumo maximas. Quantum tibi debet mycologia certe meam superat laudem et quod illa collectione me cohonestaveris ex intimo pectore apto remunerare. Sed hodie unica modo hora concessa est, intra quam, quae tibi mittenda sunt, colligenda. Excusei igitur litteras has maxime interruptas, excusei plantarum exiguum numerum speciminaque

minus selecta. Certo certius mox transmittam amplam plantarum collectionem tam phanerogamarum, quam fungorum. Hac vice modo sequantur:

(1) Musci paucissimi.
(2) Lichenes rariores, desiderati 1 num.
(3) Scleromyc. Suec. Dec. I–XVIII, XIX–XXII, XXVII–XXX. Qui desidaruntur proxime mittam. Excusei, precor, quod hodie completa specimina mittere non valeo.

Plantas omnes americanas spontaneas, praecipue Lichenes et Phanerogamas, avidissimas desidero. Taceo fungos, non minus gratos. Observationes, quas communicare promiseris mihi certe perquam utiles erint. In Suppl. Syst. Myc. elaborando de loco Agaricorum Americanorum plurimorum dubius sum.

Secundum volumen Systematis Mycologici etiam grato amico hac vice exfero. Plures libros recentiores alia vice.

Finem imponam harum litterarum, quaram brevitatem et negligentiam iterum iterumque excusei precor. Summa temperi angustia ita me prevenit, ut tantum liceat stante pede quaeque in mentem veniant conscribere. Interea me in favore et amicitia tua inclusum habeas. Valeas.

Lundae in Suecia d. xv Apr. 1824.

To the most distinguished, most illustrious Dr. Schweinitz
Elias Fries sends most hearty greetings.

I have received with pleasure your very kind letter and the valuable collection of plants sent by the kindness of Liliencron, and express the greatest possible thanks for them. How much mycology owes to you is surely beyond my praise and I sincerely hope to return the favor of this collection with which you have honored me. But today I have only an hour in which to collect what is being sent to you. Therefore excuse this much interrupted letter, the small number of plants, and the poor selection of specimens. Very surely I will soon forward a large collection of plants, phanerogams as well as fungi. This time there are only the following:

(1) A very few mosses.
(2) More rare lichens, 1 number lacking.
(3) Scleromyc. Suec. Dec. I–XVIII, XIX–XXII, XXVII–XXX. The missing numbers I will send very soon. I beg you to excuse me for not being able to send the complete set today.

I am very eager to have any American wild plants, especially lichens and phanerogams. I do not speak of the fungi, which are no less welcome. The observations which you promise to communicate to me will certainly be exceedingly useful to me. I am

doubtful as to where to place very many of the American agarics in the Supplement to the Systema Mycologicum.

This time I am also sending to my valued friend the second volume of the Systema Mycologicum. Several more recent books at another time.

Let me close this letter, the brevity and carelessness of which I beg you again and again to excuse. The extreme shortness of time prevents me from being able to write at the moment what I have in mind. Meanwhile keep me in your favor and friendship. Farewell.

Lund, Sweden, Apr. 15, 1824

THE GENUS GOMPHIDIUS FRIES IN NORTH AMERICA

Rolf Singer

(with 3 figures)

DESCRIPTION OF GENUS

Pileus glabrous to tomentose or fibrillose, viscid or more rarely dry; hymenophore lamellate, consisting of rather thick, decidedly decurrent gills with frequently rather obtuse edges and waxy-gelatinous consistency; lamellae moderately close, subclose to distant, arcuate, later descendant, gray to fuliginous when mature; spore print between "bone brown" and black, usually fading out to deep rusty brown in old spore prints; spores melleous to gray, fusoid to ellipsoid-cylindric, smooth, with thin to slightly thickened but simple non-amyloid walls and without a germ pore; sterigmata usually with a very broad base, mostly four on a basidium; basidia voluminous and variable in length; cystidia present on the edges and on the sides of the lamellae, often appearing more scattered in old specimens because of the collapsing of the walls, sometimes slightly colored but more often hyaline except for a colored resinous incrustation, large, thick- or thin-walled, originating in the subhymenium; trama thick, consisting of an often rudimentary mediostratum, a lateral stratum of non-divergent hyphae, and an irregular hymenopodium which is rather indistinctly limited from a filamentous-intermixed or filamentous to filamentous-cellular subhymenium; hyphae without clamp connections; stipe frequently with a yellow lower portion; context thick in the middle of the pileus and at least in some parts of it (especially under the cuticle) strongly reacting with most of the usual inorganic reagents; veil present, cortinoid or glutinous-hyaline, rarely glutinous and fugacious at an early stage; mycelium white or colored, forming mycorrhiza with conifers. This genus has its natural affinities with the Boletineae.

Type species: *Gomphidius glutinosus* (Schaeffer ex Fr.) Fr.

A. Context of the pileus ochraceous to orange (though at times in young specimens rather pallid), more rarely salmon to pink; veil constantly present, consisting of strictly parallel, pigment-incrusted hyphae, macroscopically never entirely glutinous, never hyaline; subhymenium filamentous-intermixed and dense to very dense; mediostratum rudimentary in young specimens, indiscernible in adult ones. . Subgenus I. Chroogomphus
> B. Pileus dry to subviscid in wet weather, not shining in dry weather, more or less tomentose to fibrillose . . . . Section *Floccigomphus*
> B. Pileus viscid in wet weather, shining in dry weather, not tomentose and not fibrillose except for traces of the veil on the margin.
> > Section *Viscogomphus*

A. Context of the pileus white, more rarely partly more or less salmon, or becoming so on exposure; veil absent, or consisting of subparallel-subinterwoven, thin hyphae which are not incrusted by pigment, microscopically hyaline to white, and often partly or entirely glutinous, sometimes gradually blackening in age; subhymenium filamentous-cellular to filamentous, moderately dense; mediostratum of young specimens distinct, less distinct in older ones.
> B. Veil visible only in the primordium, fugacious and not leaving any traces in adult specimens; dermatocystidia of the stipe fasciculate and the fascicles forming glandulae which make the stipe appear fibrillose or furfuraceous (though these macroscopical characters not always very obvious); mycelium (of *G. maculatus*) connected with *Larix*-mycorrhiza . . . . Subgenus II. Laricogomphus
> B. Veil covering the lamellae of young specimens and leaving more or less distinct traces even in old specimens; dermatocystidia of the stipe not fasciculate; no fibrils or glandulae made up of dermatocystidia present on the surface of the stipe; mycelium in nature not connected with larch but rather forming mycorrhiza with a variety of other conifers (*Pinus, Picea, Tsuga, Pseudotsuga, Abies*).
> > Subgenus III. Myxogomphus
> > C. Spores (with exception of immature and aborted ones) longer than 14 μ . . . . Section *Macrosporus*
> > C. Spores, or majority of spores, of a mature carpophore, shorter than 14 μ, or just reaching 14 μ . . . . Section *Microsporus*

Subgenus Chroogomphus Singer

Papers Michigan Academy Science Arts and Letters **32** : 150.   1948

Section *Floccigomphus* Imai

Journ. Agr. Hokk. Imp. Univ. **43** : 285.   1938

A. Cystidia thick-walled (walls 1–4 μ thick), never collapsing, numerous in old specimens . . . . .1. *G. tomentosus*
A. Cystidia thin-walled (walls up to 1 μ thick), collapsing in poorly dried or very old material and then not readily demonstrated . .2. *G. leptocystis*

1. Gomphidius tomentosus Murrill
Mycologia 4: 307. 1912

Pileus fleshy, thick, abruptly thin at the incurved margin, ovoid-subhemispheric to convex when young, later convex and expanding, in age often plane and with uplifted margin, the disc often conical at first, later often umbonate, more rarely umbilicate or depressed around a papilla, 20–100 mm. broad, surface innately and appressedly tomentose, moist to somewhat sticky-subviscid in very damp weather, not becoming scaly except in age and then because of the breaking up of the surface layer or because of the disintegrating of the tomentum into tomentose, strictly appressed squamulae, disc sometimes rugulose or uneven, margin fringed at first with the fibrillose remnants of the veil, uniformly "ochraceous buff," or "capucin buff," or "pale yellow orange," or "light ochraceous salmon," or "light ochraceous buff," or "pinkish buff" (the color depending on the amount of moisture present, *e.g.,* "pinkish buff" appearing with low moisture content, etc.), the fibrils sometimes assuming a slight pinkish-vinaceous tinge, especially in age when the disc tends to become "zinc orange"; flesh about "ochraceous orange" or "capucin buff," or "ochraceous buff," or cortex near "capucin orange," unchanging, with KOH and NH_4OH purple, with $FeSO_4$ entirely olivaceous gray, with formol negative, soft and rather pliant when fresh, odor slight, agreeable, or none, taste slight, tardily slightly disagreeable; lamellae decurrent, inserted, subclose to distant, a few forked, broad but narrow in small specimens (3.5–12 mm.), often wrinkled or venose near the margin, concolorous with the pileus, then sooty from the spores; spore print about "bone brown" to nearly black; spores (16.5) $17–24.5 \times 6.5–9\,\mu$, most frequently $18.5–20.2 \times 7.5–8.2\,\mu$, ellipsoid, pale sordid-melleous, some deeper colored, comparatively broad; basidia $40–56 \times 11–13.5\,\mu$, 4-spored; cystidia $136–242 \times 14–24\,\mu$, ventricose, capitate, or cylindric, sometimes with narrowed apex which, however, is always rounded, the walls thick ($1–4\,\mu$) but in a few cystidia sometimes thin, their outer surface often incrusted by a fulvous-castaneous to melleous resinous incrustation, their inner surface also sometimes colored or the lumen filled with a colored sap, numerous on both the sides and edges of the lamellae and never collapsing; subhymenium filamentous-intermixed, dense; trama with a slight mediostratum which soon becomes indefinite; lateral stratum with a distinct axillar arrangement of subparallel-subinterwoven hyphae, looser than the hymenopodium which is scarcely delimited from the subhymenium, often with pink areas in freshly dried material; stipe subequal or tapering gradually or abruptly at the base, or

subventricose, solid, fibrillose or loosely tomentose to innately floc-culose with scurfy apex, glabrescent, "light orange yellow" (from the veil), or concolorous with pileus, "ochraceous orange" when scratched, 40–100(180) × 5–20 mm.; context concolorous with the context of the pileus, the periphery concolorous with the scratched surface, neither bright yellow nor deep orange in the base, unchanging, rather soft, usually turning pinkish in well dried specimens; mycelium pink, the basal tomentum consisting of thin- or thick-walled hyphae 8–17 μ in diam., occasionally incrusted by a melleous or hyaline incrustation, interwoven, cylindric, and all hyphae without clamp connections.

Type locality: Seattle, Wash., New York Botanical Garden (type seen).

Habitat: Among mosses or on naked clay-soil in coniferous woods near *Pinus, Abies, Tsuga, Pseudotsuga,* etc., scattered to very gregarious up to the subalpine zone, fruiting from September to November.

Distribution: Washington to California (gradually becoming more uncommon toward the south), also in Hokkaido and Honshu, Japan.

Note: A. H. Smith has collected a subalpine form with stouter stipes (30–60 × 10–30 mm.) but otherwise identical with the type form. *G. tomentosus* sensu Kauffm. is *G. tomentosus* Murr. plus *G. leptocystis. G. tomentosus* sensu Humbolt is *Cystogomphus Humboltii* Sing.

2. Gomphidius leptocystis Singer (Figs. 1–2)
Papers Michigan Academy Science Arts and Letters **32** : 148.   1948

Pileus fleshy, thick, abruptly thin at margin, campanulate, oval-hemispheric, soon expanding, convex then plane, or somewhat depressed, obtuse or more rarely with an acute or obtuse umbo, 30–90 mm. broad, surface strongly felty-fibrillose when young, later the felt breaking up to form hairy scales on the disc and around it where the dried pileus assumes a squarrulose appearance, less so on margin, the hairs "ochraceous buff" on "buckthorn brown" ground, sticky-subviscid and "vinaceous tawny" to "wood brown," or "drab" near the margin and more yellowish toward the disc in very damp weather, "ochraceous orange" to "ochraceous buff" in dry weather, tending to assume a "deep brownish vinaceous" ground color and darker (to "warm blackish brown") spots and fibrils giving the general impression of about "Prussian red," "deep

livid brown," "Mars violet" where wounded or when freshly dried; this purple color due to a purple incrustation of the hyphae which turns melleous in KOH; the pellicle separable from the context which is paler, nearly whitish, and shot through with streaks of "pinkish buff" or slightly changing to purplish in places, inodorous, taste tardily and slightly disagreeable; lamellae usually unequally

Photo A. H. Smith.

FIG. 1. *Gomphidius leptocystis.* × 1.

decurrent, inserted, close to subdistant, a few forked, often ana-stomosing, with entire edges, broad (6–13 mm.), "ochraceous buff" to "ochraceous salmon," at last "clay color," "ochraceous tawny," sooty from the spores; spore print nearly black; spores 14–20 × 6–8.2 μ, mostly about 16–17 × 6.8–7.3 μ, ellipsoid to fu-soid, pale sordid melleous, some deeper colored; basidia 38–68 × 11–13 μ, 4-spored; cystidia 120–238 × 12–21 μ, hyaline with a ful-

vous-castaneous resinous incrustation, or with a melleous granular incrustation, with thin (up to 1 μ) walls or very few occasionally up to 1.5 μ thick, the inside of the walls not colored (except in very few cystidia in occasional specimens), easily collapsing by careless preparation, in age readily collapsing and hence appearing scattered in old specimens; all hymenial bodies and even the hyphae initially with a
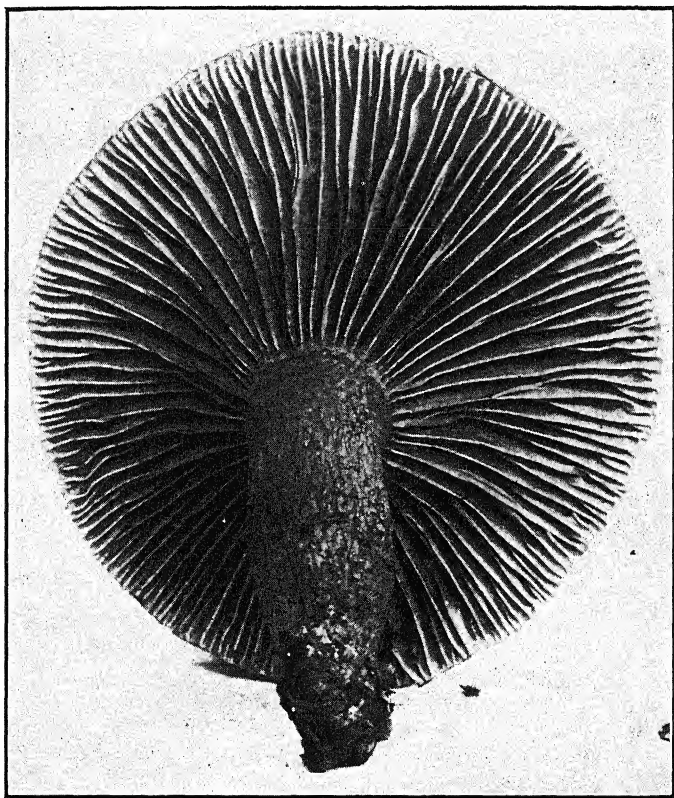
Photo A. H. Smith.

Fig. 2. *Gomphidius leptocystis.*

purple incrustation (NH_4OH) when freshly dried, the incrustation becoming melleous in alkali; trama and subhymenium as in *G. tomentosus;* stipe subcylindric or attenuated below, sometimes fusiform, mostly rather long, occasionally short, fleshy solid or hollowed by grubs in age, floccose then lacerate-floccose, the floccosity initially continuous with that of the pileus, the lower portion often denudate by being deeply imbedded, apex with slight remnant

of the fibrillose-silky "buff yellow" to "maize yellow" veil, otherwise colored as the pileus and becoming purplish by handling and drying, downward becoming "empire yellow" or sordid ocherbrown, 60–130 × 8–25 mm.; context in the upper portion concolorous with that in the pileus, in the base "empire yellow," rather hard and rigid; mycelium pinkish; all hyphae without clamp connections.

Type locality: Lower Stony Creek, Garibaldi, B.C.   J. E. Davidson 222 (type at Michigan University Herbarium).

Habitat: Mostly in deep needle beds and on humus, in mixed coniferous woods under or near pines, scattered to gregarious, fruiting from August to October.

Distribution: Northwestern part of North America: British Columbia to Oregon and East to Idaho.

Illustration: Kauffman, Mycologia 17, pl. 14. 1925 (as *G. tomentosus*).

Note: This species differs from *G. tomentosus* in the characters of the covering of the pileus, in the pigment, in the thin walls of the cystidia, and in somewhat smaller average spore size.   It differs from the closely related *G. sibiricus* in the color of the base of the stipe which is "empire yellow" instead of deep orange.

<div align="center">

Section *Viscogomphus* Imai

Journ. Fac. Agr. Hokk. Imp. Univ. 43 : 284.   1938

KEY TO SPECIES
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A. Mycelium pink or pallid-salmoneous.
  B. Cystidia thick-walled in their middle portion ....3. *G. vinicolor*
  B. Cystidia thin-walled throughout ....4. *G. ochraceus*
A. Mycelium Isabella color, or melleous, or light to bright yellow; cystidia thin-walled throughout ....5. *G. rutilus*

<div align="center">

3. GOMPHIDIUS VINICOLOR Peck

N. Y. State Mus. Rep. 51 : 291.   1898
</div>

Pileus usually thick and fleshy at least in the center, thinning out toward the margin, conic-convex, then convex, usually umbonate, or at least the majority of the pilei of a group umbonate, 10–90 mm. broad; surface viscid, shining when dry, glabrous, naked, smooth, rarely diffracted in age, ochraceous gray or between "Army brown" and "Natal brown," becoming deep reddish brown ("Mikado brown," "chestnut," "liver brown," "carob brown"), eventually of-

ten deep vinaceous red; pellicle consisting of repent, subparallel-subinterwoven, fulvous-castaneous-incrusted, elongate hyphae; context pale ochraceous to "pale ochraceous salmon," sometimes very slightly turning pinkish on injury, sometimes umber immediately beneath the pellicle, with KOH between "pale rosolan purple" and "thuilite pink," and becoming "spinel red," with NH_3 vapors "liseran purple" and "pale rosolan purple," with $FeSO_4$ "deep olive" to "dark olive" or more olive gray; with formol negative; lamellae initially dirty ochraceous-alutaceous, becoming between "Prout's brown" and "mummy brown," eventually almost black from the spores, broadest in the middle (4–9 mm., *i.e.*, broad), decurrent, arcuate, or arcuate-plane, inserted, slightly transversely venose, or smooth, only rarely and very sparsely forked, subdistant to distant; spore print from "clove brown" or "olive brown" to nearly black; spores (17) 18.5–26 × 6–7.5 μ, melleous-pallid to melleous grayish, or melleous, ellipsoid to cylindric or fusoid; basidia 45–61 × 12–15.7 μ, 4-spored; cystidia 108–177 × 13.6–19 μ, numerous though not unusually crowded, on the edges as well as on the sides of the lamellae, strongly incrusted on the outside by a castaneous-fulvous resinous mass, and slightly incrusted in some cases on the inside of the walls which are thin to moderately thick at the apex and thin at the very base but distinctly thickened (1.3–5.8 μ) either in the middle or just below the middle, hyaline, cylindric or slightly ventricose below; subhymenium filamentous-intermixed, very dense and irregular; mediostratum of very few, parallel, cylindric hyphae, indistinct or soon disappearing; lateral stratum less dense and more hyaline, subirregular with an axillar arrangement but becoming more and more irregularly interwoven with age; hymenopodium only slightly distinct from the subhymenium, strongly colored; stipe subequal or gradually tapering downward, not viscid, glabrous except for the veil, smooth but sometimes grooved when drying, naked, sordid ochraceous to "light vinaceous cinnamon" to "buff pink," or "vinaceous rufous," the base mostly "cinnamon" to "clay color," "light ochraceous buff" or "ochraceous buff," sometimes at places becoming "orange rufous" to almost "Jasper red" or "orange cinnamon" or "cacao brown," sometimes with blackish spots on handling, solid, 27–95 × 3–14 mm.; veil either forming a definite fibrillose-woolly narrow annulus which is concolorous with the remaining surface, or reduced to a mere fibrillose zone at the apex of the stipe and consisting of strands of parallel, incrusted hyphae; mycelium pinkish; context similar in color to that of the pileus but either more "clay color" or more "primuline yellow" at the base in many specimens; odor none or very slight, agreeable; taste mild; the context of the base turning olive with NH_3, brownish with NH_4OH, deep fulvous-yellow with H_2SO_4 (if dried subsequently),

olive as in the pileus with $FeSO_4$; all hyphae without clamp connections.

Type locality: Lake Mahonk, N. Y., U. S. A.. (New York State Museum, type seen).

Habitat: Under *Pinus Murrayana, P. resinosa, P. rigida, P. virginiana,* or near one of these species in mixed woods, on the ground, gregarious, fruiting from August to November.

Distribution: From Maine to Tennessee and from Ontario across the continent to British Columbia, Washington, Oregon and the mountainous part of Northern California.

Note: This is considered as ssp. *typicus* of the species. Peck's types are this. It is the northern race of the complex species consisting of geographic and ecologic races. It differs from ssp. *jamaicensis* in slightly more distant lamellae, slightly larger spores, and the presence, in most specimens, of an umbo; it differs from ssp. *californicus* in the smaller number of cystidia present, and also in the presence of an umbo and more distant lamellae; all these races differ in their geographic areas and the pine species with which they form mycorrhiza in nature.

Ssp. JAMAICENSIS (Murr.) Sing., Farlowia 2 : 531.   1946

Gomphidius jamaicensis Murr., Mycologia **10** : 69.   1918.
Gomphidius alachuanus Murr., Journ. Elisha Mitch. Soc. **55** : 367.   1939.

Pileus consistently exumbonate in mature specimens, never red, 24–98 mm. broad; lamellae subclose to subdistant; spores 17–21.8 × 5.8–8 μ, melleous grayish to gray; basidia 34–46 × (8.3) 9.2–13 μ; 4-spored; cystidia 115–150 × 13.5–22.5 μ, thick-walled in the middle; stipe 36–100 × 4–15 mm., rarely becoming black on handling; all other characters practically identical with those of the type subspecies. Habitat: Under *Pinus taeda,* and probably also under *P. caribaea, P. australis,* and *P. palustris,* on the ground in sandy woods, or near these pines in mixed woods, gregarious, fruiting from November to January; North Florida and Alabama, also in the Mountains of Jamaica.

Ssp. CALIFORNICUS Singer, Pap. Mich. Acad. **32** : 149.   1948

Pileus obtuse or subumbonate, more rarely distinctly umbonate, usually rather large to very large; lamellae subclose to subdistant;

cystidia extremely crowded and almost as numerous as the basidia; all other characters including the spore size exactly as in the typical form. Under or near *Pinus radiata* and *P. ponderosa* on soil preferably rich in nitrogen, often subcespitose, from late fall until spring in California.

4. GOMPHIDIUS OCHRACEOUS Kauffman ssp. TYPICUS Mycologia
17: 119. 1925

Pileus fleshy, abruptly thin at the margin which is at first incurved, convex, then becoming plane, often with decurved margin and the disk slightly depressed around the umbo, but just as often without any umbo or with a very indistinct one, 15–75 mm. broad; surface viscid, shining when dry, glabrous, smooth or sometimes rugulose from the drying gluten, "ochraceous salmon," "apricot orange," "deep chrome" on the margin when fresh, "zinc orange" to "ochraceous orange" elsewhere but sooner or later more or less clouded with "olive brown" or grayish in the center, then also near the margin and gradually becoming some tint around "vinaceous tawny" or plainly vinaceous, "Indian red" to "dark Indian red" in recently dried specimens; pellicle thick and toughish, separable; context whitish with a tinge of "pinkish buff" to evenly "light ochraceous buff," with KOH and NH_4OH pinkish to purple, with $FeSO_4$ greenish-gray and finally inky-violaceous, with formol negative, odor none, taste mild; lamellae decurrent, close to distant, inserted, rather broad (about 7–8 mm. in larger caps), "tawny" or ochraceous, soon sooty from the spores; spores 17–20.5 (22) × 6.5–7.3 (8) μ, fusoid-ellipsoid, pale sordid melleous, some deeper colored; basidia 47–56 × 11–15 μ, 4-spored; cystidia 116–185 × 11–16.5 μ, very numerous on edge and sides, cylindric or ventricose, thin-walled (walls up to 1 μ thick not counting the incrustation), hyaline with strong hippocastanus-brown resinous incrustation; subhymenium, hymenopodium, lateral stratum and mediostratum as in *G. leptocystis;* stipe tapering downward, slightly curved or flexuous, more rarely straight, "orange buff," or concolorous with some part of the pileus, at last partly vinaceous, flocculose-fibrillose to an obsolete cortinoid annulus and this fibrillosity with a strong tendency to become subviscid to viscid on its outer side in a humid atmosphere but soon drying out in drier weather, 30–185 × 4–20 mm., veil concolorous with the rest of the stipe, consisting of parallel hyphae which are strongly incrusted by pigment; context concolorous with the surface; mycelium pink; all hypae without clamp connections.

Type locality: Mt. Hood, Ore., University of Michigan Herbarium (type seen).

Habitat: Under conifers (*Pinus monticola, Abies* spec., *Pseudotsuga, Tsuga,* probably usually connected with *Pinus monticola*), mostly gregarious, fruiting from September until later in fall.

Distribution: From Washington to Northern California.

Note: The above description is given for the Western race with the yellow-ochraceous-orange margin of the pileus and somewhat orange stipe. It is not only the nomenclatorial type but probably also the form most closely related to the one from which all American subspecies originated, and on the other hand, more closely related to the species of the preceding section than any other species or race in this section.

Ssp. **muscigenus** Singer * ssp. nov.

Pileus moderately fleshy, constantly with a small acute umbo, 15–50 mm. broad; surface "bay" or "auburn," or "tawny," or "Hay's russet," or between "buffy brown" and "olive brown," with the margin often more yellowish brown (*e.g.,* "ochraceous tawny" or more dingy then "salmon buff"); lamellae macroscopically and anatomically as well as the spores not different from the type of *G. ochraceous;* stipe "ochraceous orange," mixed with "ochraceous tawny" and "light buff," or "capucin buff" to "capucin orange," becoming "apricot orange" or "light salmon orange," 35–80 × 4–10 mm.; mycelium "flesh pink"; context concolorous with the surface or more yellow below ("Naples yellow," slightly tending toward "warm buff" and "massicot yellow") but between "pale pinkish buff" and "pinkish buff" at the very base; otherwise like ssp. *typicus.* Under or near pines of the subgenus *Haploxylon,* very often in sphagnum-swamps or otherwise in deep moss beds, but also in deep needle beds and on very decayed wood; from Maine and Ontario to New York and West to Wyoming; Icon. Farlow. pl. 70 (*G. viscidus* var. *testaceus*); usually associated with *Suillus americanus.*

Ssp. **superiorensis** (Kauffm. & Smith) Singer comb. nov.

G. superiorensis Kauffman & Smith, Pap. Mich. Acad. Sci. 17: 170. 1933.

Pileus "Natal brown," "army brown," also "pecan brown"; stipe and cortina "ochraceous buff," otherwise like ssp. *muscigenus.*

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* A typo colore pilei haud ochraceo-aurantiaco et probabiliter associatione mycorhizina differt.

Under *Pinus resinosa* and possibly other pines of the subgenus *Diploxylon* in sphagnose places in Michigan. Illustration: Kauffman & Smith, Papers Mich. Acad. Sci. 17, pl. 30.

5. Gomphidius rutilus (Schaeff. ex Fr.) Lundell & Nannfeldt Fungi Exsicc. Suec. 409. 1937

Agaricus rutilus Schaeff. ex Fr., Syst. Myco. 1: 315. 1821.
Cortinaria rutila S. F. Gray, Nat. Arr. Brit. Pl. 2: 629. 1821.
Gomphidius viscidus (L. ex) Fr., Epicr. p. 319. 1838.
Gomphidius testaceus (Fr.) Britz., Hymen. Sudb. 4: 133. 1885 (a var.).
Gomphidius litigiosus Britz., Hymen. Sudb. 9: 14. 1893 (a var.).
Gomphidius alabamensis Earle (Nom. subnud.) (ssp. *alabamensis* Sing.).
Gomphidius viscidus var. *columbiana* Kauffm., Mycologia 17: 122. 1925.

Pileus conic to campanulate with incurved margin, then expanding, with umbo or rarely without umbo, the margin often eventually uplifted, 15–60 mm. broad; surface with a fibrillose "apricot buff" veil-zone on young caps, otherwise glabrous, smooth but frequently cracking in age and becoming scaly, viscid except on the veil-zone, soon entirely viscid in wet weather but not glutinous, usually "olive brown" when quite young, later with yellow tints, becoming "vinaceous tawny" to "Rood's brown" or "Hay's russet" when old, at last sometimes reaching "light russet vinaceous" or even "Indian red," with H_2SO_4 pale brown (almost no reaction); context pale brownish buff, or more reddish, sometimes sordid-pallid, with KOH lilac, with NH_3 violet then violet blue, with $FeSO_4$ olive-gray to deep greenish black; odor none or slight, fruity; lamellae "ochraceous tawny" or paler when young, then "vinaceous brown," "Prout's brown," "Saccardo's umber," "tawny olive," or "sepia," subdistant to distant, simple or very few forked, broad (5–8 mm.), inserted, decurrent; spore print between "clove brown" and black; spores (15) 18.5–23 × (5) 6–7.8 μ, ellipsoid-elongate to subfusoid, or subcylindric, sordid-melleous; basidia 40–54 × 14–15 μ, 4-spored; cystidia 60–170 × 12–21 μ, numerous on edges and sides of the lamellae, thin-walled throughout, subcylindrical, ventricose or capitate, with a strong resinous incrustation of mahogany-brown to hippocastaneous-brown, more rarely honey color; subhymenium filamentous-intermixed and very dense, appearing irregularly cellular when looked upon superficially, colored; mediostratum axillar, of loose, parallel hyphae, indistinct from the beginning and soon entirely obliterated; lateral stratum non-divergent, of subparallel-interwoven hyphae, looser than the hymenopodium which is not much different from the subhymenium

but having mostly somewhat larger elements than the latter; stipe "ochraceous buff," pallid at the very apex, at least initially, but soon concolorous or "Buckthorn brown," or covered with colored fibrils or with the spores, fibrillose from the veil which gives it a "flesh ocher" to "apricot orange" color, or becoming pinkish red with age, dry and equal or tapering downward, solid, often becoming somewhat hollow from grubs, often slightly compressed or wavy and flexuous, 30–80 × 6–15 mm.; mycelium "honey yellow," "Isabella color," or "Naples yellow," "mustard yellow," "primulin yellow," or mixtures of these and white; context of the stipe concolorous with the flesh of the pileus in the upper portion, *i.e.*, a pale yellowish buff, often becoming "apricot orange," richer yellow toward the base; all hyphae without clamp connections.

Type locality: Regensburg, Bavaria (type specimens not preserved).

Habitat: On bare soil and among needles, also among mosses but usually not in sphagnetum, accompanying pines of the subgenus *Diploxylon,* in this Hemisphere excusively with pines of the series Insignes (*e.g., Pinus Murrayana, P. radiata*), fruiting from summer until late fall, or spring (in California).

Distribution: From the Rocky Mountains to the Pacific Coast, also in Europe and Northern Africa, Northern Asia, east to Hokkaido and Honshu, Japan.

Illustrations: Represented in almost all European mushroom books and illustrated scientific manuals, best in Lange, Flora Agar. Dan. 5; Kavina, Ceske Slizaky, Trav. Mycol. Tchecosl. 2, fig. 8, etc. (see Saccardo, Syll. **19**: 789 sub *Gomphidius viscidus*).

Exsiccata: Saccardo, Mycotheca Veneta 810; Roumeguere, Fungi Gallici 3815; Fuckel, Fungi Rhenani 1429.

Note: The above described form includes the American plant which Kauffman distinguished under the name var. *columbiana.* The European form grows with pines of the series Lariciones while the American form is found near pines of the series Insignes. Except for this physio-ecological difference, which by the way escaped Kauffman because he had no clear conception of his variety, we are unable to confirm any of the minor differences enumerated and discussed by Kauffman. The type of var. *columbiana* is identical with the European *G. rutilus* of which we distinguish only a ssp. *typicus* as described above, with var. *typicus,* var. *testaceus,* and

other varieties of European importance only, and ssp. *alabamensis* occurring in the Southern States of this country:

Ssp. ALABAMENSIS Earle ex Singer, Farlowia 2: 535.   1946

This differs from the type subspecies in the absence of an umbo (at least in the vast majority of mature specimens), the more furfuraceous than fibrillose apex of the stipe, and the more frequently · bright yellow base of the stipe, and perhaps some minor characters of doubtful constancy.   It grows near pines of the series Australes and Insignes from Maryland to Northern Florida, and west to Tennessee and Alabama.   The type was collected by Earle in Alabama but is here considered as a nomen subnudum, published in a key to the species of *Gomphidius* but never described properly.

SUBGENUS LARICOGOMPHUS Singer
Papers Mich. Acad. Sci. Arts and Letters 32: 149.   1948

KEY TO SPECIES

A. Pileus "vinaceous pink" when young; spores 23.5–32 × 7.5–8.2 μ.
$$7.\ G.\ flavipes$$
A. Pileus more brownish than "vinaceous pink," or whitish when young; spores 18–25 × 7–9 μ ....6. *G. maculatus*

6. GOMPHIDIUS MACULATUS (Scop. ex Fr.) Fries
Epicr. Syst. Myc. p. 319.   1838

Agaricus maculatus Scop. ex Fr., Syst. Myco. 1: 315. 1821.
Cortinaria viscida atropuncta Gray, Nat. Arr. Brit. Pl. 2: 629. 1821.
Gomphidius gracilis Berk. & Br. apud Berk., Outl. Brit. Fungology, p. 196. 1860.
Gomphidius maculatus var. *gracilis* Quél., Enchir., p. 91. 1886.
Gomphidius furcatus Peck, Bull. N. Y. State Mus. 5: 649. 1899 (a variety).
Gomphidius maculatus f. *gracilis* Kavina, Trav. Mycol. Tchec. 2: 5. 1924.
Gomphidius maculatus ssp. *gracilis* Konr. & Maubl., Icon. Sel. Fung. 4: pl. 329, 6: 442. 1924–1938.
?*Gomphidius stillatus* Strauss in Sturm, Deutschl. Flor. III, 33–34: 3. 1853 (see also "Doubtful species").

Pileus convex, eventually plane or irregularly depressed, umbonate or obtuse, 20–110 mm., surface viscid to glutinous, glabrous and smooth, rarely with some slight pallid fibrils in young specimens, dull fuscous pink to reddish brown in varying intensity, most

frequently "light pinkish cinnamon" to "orange cinnamon," but sometimes almost pallid or whitish and often later assuming a reddish tone because of the reddening of the underlying context, reaching "dragon's blood red" or "Hay's russet," usually becoming stained with black more readily than other species; hyphae of the cuticle incrusted, 4–14 μ thick, a few terminal members cystidium-like and obtusely rounded at the ends, olive colored in NH_4OH; context white, lemon yellow toward the base, when injured reddening in the white portion to pink, latericious, or almost wine red, but the reddening often failing to develop in dry weather or in too watery material, with a watery, then brick red line just beneath the cuticle; odor none, or of cumarin; taste mild; context becoming dark green with $FeSO_4$, intensely brick color with NH_3 and NH_4OH; with formol lilac-red; lamellae short decurrent to decidedly decurrent, usually many-forked, subdistant to distant, variable in breadth, pallid, then gray, often somewhat reddening; spore print black or nearly so; spores 18–25 $\times$ (6) 7–9 μ, dark brownish gray, some paler, subfusoid; basidia 38–66 $\times$ 10–17 μ, 4-spored; cystidia (100) 133–216 $\times$ 16–26.5 μ, numerous, fusoid, rounded above, the apex attenuate or cylindrical, mostly filled with a gray sap, covered with grayish brown to dark gray incrustations, strongly (80–100 μ) projecting, thin-walled; subhymenium filamentous or filamentous-subcellular, rather loose, mediostratum moderately distinct in very young specimens, later becoming rather indistinct; stipe immensely variable in shape, pallid whitish, or reddish pallid, intensely yellow to lemon yellow at the base, mostly covered with reddish bay glandulae which sooner or later become blackish, tending to blacken partly or entirely by pressure or on drying, beset with colorless watery droplets at the apex in many specimens, the droplets becoming brownish or reddish after a short time, but in dry weather the glandulae often inconspicuous and covering only a narrow zone of the stipe, solid, 40–80 $\times$ 3–35 mm.; glandulae consisting of fascicles of dermatocystidia, dermatocystidia olive black in NH_4OH, little or not incrusted, about 100 μ long and 8–13.5 μ thick, cylindric; context concolorous with the surface and otherwise as in the pileus; mycelium whitish to yellowish; all hyphae without clamp connections.

Type locality: Carniola (type not preserved).

Habitat: In larch woods and tamarack swamps (in America mostly near *Larix laricina* = *L. americana*), associated with Suilli of the section Larigni (*S. aeruginascens, S. Grevillei* var. *Clintonianus* and others), fruiting from July to October.

Distribution: From New England to North Carolina and west to Michigan and Ontario, possibly also farther west and north, but infrequent at most localities; also in Europe and Asia (as far as the area of *Larix* extends).

Illustrations: Kauffman, Agar. Mich. 2, pl. 23; Cooke, Illustr. pl. 883; Lucand, Champ. Fr. pl. 346 A–B; Ricken, Blätterp. 2, pl. 3, fig. 2; Bresadola, Icon. Myc. 14, pl. 675. The remaining figures are rather poor: Berkeley, Outl. pl. 12, fig. 7; Britzelmayr, Hym. Sudb., Gomph. fig. 9, 11, 12; Cooke, Illustr. pl. 882; Bresadola, Iconogr. 14, pl. 674; Konrad & Maublac, Icon. 4, pl. 389.

Note: Eurasiatic specimens more often have broader than narrower spores, while in the American specimens this situation is reversed.

Var. **furcatus** (Peck) Sing. comb. nov. (*G. furcatus* Peck, l.c.). Base of the stipe not bright yellow. Type from Kasoag, N. Y., distribution unknown.

7. Gomphidius flavipes Peck
N. Y. State Mus. Rep. 54: 153. 1901

Pileus convex, expanded and repand in age, obtuse or subumbonate, 15–40 mm. broad; surface even, viscid, the viscidity soon slight, with a thin, minutely tomentose (?) pellicle with silky fibrils on the margin, "vinaceous pink" when fresh, becoming duller in age; lamellae arcuate, subdecurrent, distant, very few forked, thick, "pale pinkish buff," at last sprinkled by the spores, 4–5 mm. broad midway between margin and stipe, often transversely wrinkled, edge entire; spores (20.5) 22.5–32.5 × (6) 7–8.2 μ, fusiform, more rarely clavate, fuscous; basidia 48–58 × 11.2–15 μ, 4-spored, very few 2-spored; sterigmata 8–10.5 μ long; cystidia 90–136 × (10) 15–20.5 μ, numerous, subcylindric or slightly ventricose, broadly rounded at the apex, hyaline; subhymenial and tramal characters as in *G. maculatus;* stipe equal above, tapering below to a pointed base, silky or lacerate-silky at apex, slightly fibrillose downward, white above, "picric yellow" or bright below (the yellow often reaching to the apex) both inside and out, solid becoming soft, sometimes curved or twisted, 50–80 mm. long; fibrils of the upper portion of the stipe consisting of fascicles of dermatocystidia which are either of the same shape as the hymenial cystidia, or more clavate, ventricose, etc., mostly hyaline; odor none; taste mild (macroscopical characters from Kauffman & Smith).

Type locality: Westport, N. Y., State Museum (type seen).

Habitat: In sphagnose bogs (the accompanying conifers unknown), fruiting from August to September.

Distribution: From New York to Michigan.

Illustrations: Peck, l.c., pl. I, fig. 1–4.

Note: This rare species may be a variety of *G. maculatus* but its glandular fibrils do not blacken and its dermatocystidia are hyaline, its spores are notably longer (the longest in the genus), and the color of the fresh pileus is more pink. The species has been placed in *Laricogomphus* tentatively, without regard to the lack of information on the tree symbiont and the reactions of the context.

Subgenus Myxogomphus Singer
Papers Mich. Acad. Sci. Arts and Letters 32: 149. 1948

Section *Macrosporus* Singer
Papers Mich. Acad. Sci. Arts and Letters 32: 149. 1948

KEY TO SPECIES

A. Veil essentially fibrillose, or partly fibrillose and partly glutinous, in the latter case the glutinous portion manifesting itself in the mature specimen by forming 1–3 slimy belts at the apex of the stipe but never forming a wide glutinous sheath; context salmon color in fresh and dried specimens, except for the lower portion of the stipe which is deep brilliant yellow ....8. *G. septentrionalis*

A. Veil essentially glutinous, or merely the inner layer fibrillose, the glutinous portion forming a conspicuous slimy sheath that covers most of the surface of the stipe and terminates, toward the apex, in a glutinous annular thickening of the slime layer, or in a crown of very fine, very narrowly distant silky fibrils which gelatinize in prolonged wet weather; context white or partly becoming slightly pinkish in age, or turning pinkish on injury, especially in the upper (subhypodermial) zone; base of the stipe either white or yellow.

    B. Eastern species with concolorous (white) base, associated with white pine (*Pinus strobus*); a substantial percentage of the cystidia and subhymenium in most specimens strongly colored gray; pileus at first whitish, then pink; pileus and stipe usually rather small and thin.

                        9. *G. nigricans*

    B. Western species, or species with very strongly and brightly colored yellow base.

        C. Cystidia of recently dried specimens, if treated with formaline, and afterwards mounted in KOH, frequently pinkish, or at least the incrustations of the elements of the hymenium partly or entirely deep pink to purplish red; context of the pileus (subhypodermial

zone) and sometimes strongly in the base of the stipe turning
pinkish to red on injury; pileus pale purplish vinaceous to pale
grayish vinaceous, or colored in similar cold, pallid hues, pallescent
in age ....10. *G. Smithii*
C. Cystidia and incrustations not reacting as described above; context
not changing to pinkish or red in any part, or at least not becom-
ing so by autoxydation when exposed to the air; pileus either more
salmoneous-testaceous to fawn color, or more distinctly livid-
brown, not, or not strongly and constantly, pallescent.
  D. Pileus in some combination of pink; the average carpophore
  medium sized; base whitish or yellow; spores $12–22 \times 4.8–6.8\,\mu$,
  mostly $14.2–18.5 \times 5.8–6.8\,\mu$—when taken from a print; my-
  celium most frequently associated with *Abies* sp. or *Pseu-
  dotsuga*, Western species ....11. *G. subroseus*
  D. Pileus livid brown, never pink; the average carpophore rather
  tall and stout; spores $15–24 \times 5–7.5\,\mu$, mostly $15.3–20 \times 5.2–6.2\,\mu$,
  i.e., in average slightly longer and narrower than in *G. sub-
  roseus* in prints taken from American specimens; species occur-
  ring in the East as well as in the West under *Pinus* spp. and
  Picea spp., occasionally also under *Abies* and *Pseudotsuga*.
  12. *G. glutinosus*

8. Gomphidius septentrionalis Singer
Papers Mich. Acad. Sci. Arts and Letters 32: 149.   1948

Pileus obconic, flat or convex, later more or less depressed, ob-
tuse or subumbonate, 35–40 mm. broad; surface of the viscid pel-
licle glabrous, smooth, "pale ochraceous salmon" to "light pinkish
cinnamon," becoming darker, about "vinaceous cinnamon," the
disc somewhat darker, when dried "ochraceous buff" to "cinnamon"
(more fuscous if dried too fast and hot, or too slowly); context
"pinkish buff" to salmon; lamellae decurrent, subdistant, rather
broad, sometimes a few forked, white, becoming pale gray, eventu-
ally darker because of the spores; spore print black (or nearly so);
spores $15.7–22.5 \times 6–8.5\,\mu$, melleous gray, grayish fuscous, or
honey-yellow, with thin to thick $(0.8\,\mu)$ walls, subfusoid to fusoid;
basidia (29) $39–52 \times 10–13.5\,\mu$, 4-spored, very few 2-spored;
cystidia (55) $88–170 \times (12.3)$ $15.7–18.5\,\mu$, rather numerous, hya-
line, sordid-pallid, or gray, cylindric or ventricose, with rounded
apex, more rarely fusoid; subhymenium filamentous, of hyaline,
interwoven hyphae, moderately dense; mediostratum distinct in
young specimens, later not very distinct; stipe cylindric, somewhat
striate with a narrow dry apical zone which is silky-fibrillose, sub-
concolorous with the pileus above, or more rarely paler, with a
deep brilliant yellow base and with more or less blackened areas or
reticulations in some specimens, with an apical membranaceous

annulus consisting of subparallel-interwoven hyaline, nonincrusted hyphae of 2.8–6.2 μ diameter, the extreme apex of the stipe with some dermatocystidia which however do not form fascicles or glands, or sometimes the annulus indistinct or lacking, but then with 1–3 narrow glutinous belts near the apex but never broadly glutinously sheathed in its lower portion, solid, 50–85 × 5–12 mm.; context concolorous with the surface; odor none; taste mild; mycelium yellowish white; veil predominantly fibrillose but viscid on its outer side when covering the hymenophore, well developed; all hyphae without clamp connections.

Type locality: Portapique Beach, Nova Scotia, Wehmeyer, 1769. University of Michigan Herbarium.

Habitat: Under *Picea* and *Abies* among their needles in mixed (spruce-fir-birch) woods, fruiting from August to October, often associated with *Boletinus*.

Distribution: From the Maritime Provinces (New Brunswick and Nova Scotia, Canada) to Maine, U. S. A.

Notes: This and the two following species (9–10) are, of all species of *Myxogomphus,* most closely related to *Laricogomphus.*

9. Gomphidius nigricans Peck
N. Y. State Mus. Rep. **48**: 12.   1897

Gomphidius vinicolor Peck var. *minor* Kauffman, Agar. Mich. **1**: 171.   1918.

Pileus rather fleshy with very thin margin, hemispheric to convex then repand, at least the disc becoming flattened, the margin at first incurved, (10) 20–70 mm. broad; pellicle very glutinous, very strongly shining when dry or dried, entire or cracking either into small areolae or radiately, thick, separable, in the very early stages white, then more and more "pinkish vinaceous" to flesh color and turning blackish brown or quite black when old or drying; context white or concolorous with the surface in the upper portion and white below; lamellae white then becoming gray and eventually black, subclose to distant, decurrent, some (a variable percentage) forked, easily separating from the context of the pileus; spores (14.3) 15.7–20 × (4.8) 5.5–6.3 (7.2) μ, brownish, sometimes a pure deep slate gray, cylindric to fusoid; basidia 40–52 × 9–12.5 μ, 4-spored, very few occasionally 2-spored, often with a grayish cell-sap; cystidia (67) 73–105 (130) × (8) 13–17 (20) μ, very numerous in most young specimens, very scattered in old ones, thinwalled, cylindric to mostly more or less ventricose or clavate, comparatively short in most specimens, with the projecting tips covered

with a sordid melleous to brown detersible, resinaceous incrusting hood, or a granular melleous incrustation, some (often all) with a deep gray cell-sap; subhymenium slightly denser than in *G. glutinosus,* the elements however of the same structure and shape but often with a grayish cell-sap, usually filamentous; trama showing the same structure as in *G. glutinosus;* stipe whitish, the apex remaining so for a while, the lower portion soon blackening entirely, because of a slimy hyaline coating that covers almost the entire stipe except the apex and causes it to become very shiny when dry, possible occasionally reticulately blackening, the transition from white to black sometimes by intermediate colors like "dark vinaceous," the base itself not covered by an abundant mycelial tomentum and not remaining paler in the blackened specimens, (30) 40–80 × (2) 3–8 mm.; context whitish, eventually sordid blackish, at times somewhat pinkish in the base, not yellow; odor none or slight; taste mild; all hyphae without clamp connections.

Type locality: Westport, N. Y., N. Y. State Mus. (type seen).

Habitat: Under or near pines (*Pinus strobus*), fruiting in August and September.

Distribution: From Massachusetts to North Carolina, and west to Michigan and Tennessee.

Illustration: Atkinson, Stud. N. Am. Fung. fig. 50–51 (?) (see "Doubtful species").

10. Gomphidius Smithii Singer
Papers Mich. Acad. Sci. Arts and Letters 32: 150.   1948

Pileus fleshy, thinner toward the margin, convex with initially incurved margin, 30–60 mm.; surface of the glutinous pellicle glabrous and smooth, "pale purplish vinaceous" to "purplish vinaceous," varying to "pale grayish vinaceous"; context white, soft, becoming very slightly flushed with vinaceous or pink in some caps when cut; with KOH yellowish at first, finally discoloring; with NH_4OH slowly pinkish; with $FeSO_4$ instantaneously blackish in the upper part of the pileus, lamellae decurrent, subclose to subdistant, white then grayish, narrow to moderately broad, frequently forked once or twice, edge even; spore print between "clove brown" and black; spores 15–19.5 × 5.5–6.5 μ, pale melleous to grayish brown, subfusoid to fusoid; basidia 50–60 × 9–12 μ, 4-spored, their apices often covered with a resinaceous incrustation; cystidia 109–160 × 13.5–19 μ, numerous, eventually scattered, hyaline to rarely gray, purplish pink (at least many) in an alkali (15 p.c. KOH) medium if previously treated with formalin, otherwise with mel-

leous granular or scaly incrustation, ventricose or subcylindric; subhymenium and trama of the general type of *G. glutinosus;* stipe slightly enlarged downward and then often tapered almost to a point at the very base, white fibrillose beneath, entire surface whitish and finally sordid vinaceous to blackish when handled, solid within, 50–80 × 8–12 mm.; veil hyaline and glutinous, forming a hyaline, glutinous sheath that covers the lower three quarters of the stipe; context white except for yellow in the extreme base, soon distinctly pinkish in base where cut, reactions as in context of pileus; mycelium between "cream buff" and "cinnamon buff" (when dried); all hyphae without clamp connections.

Type locality: Rhododendron, Ore., A. H. Smith 19312; Univ. Mich. Herb.

Habitat: Scattered under lodgepole pines and Douglas fir.

Distribution: Oregon, U. S. A.

Note: This species, which has the colors of the fresh carpophores as well as the chemical characters including the immediate autoxidation of the context and the purplish cystidia in KOH (quite different from *G. subroseus*), is hardly distinguishable from *G. subroseus* var. *homobasis* when merely compared in the herbarium; the purplish coloration of the cystidia was absent in material of *G. subroseus* and *G. glutinosus* dried in exactly the same way and at the same time.

Var. *xanthobasis* Singer, Papers Mich. Acad. Sci. **32**: 150. 1948. Base of the stipe distinctly bright yellow. Under a plantation of *Pinus flexilis* in the old Harvard Forest, Hamilton, Mass.

11.   Gomphidius subroseus Kauffman
Mycologia **17**: 120.   1925

Pileus fleshy, abruptly thin at margin, hemispheric or convex to repand, becoming plane or broadly depressed, obtuse, 30–90 (100) mm. broad; surface glabrous, smooth, or sometimes slightly innately striate or very slightly wrinkled under the glutinous or viscid surface layer, shining when dry, "testaceous," "vinaceous fawn" (rarely reaching "fawn color"), "light cinnamon drab," "vinaceous" tawny," "flesh color," "vinaceous pink," "ocher red," "Japan rose," "salmon buff," "vinaceous buff," "salmon color," the margin concolorous or paler, becoming "onion skin pink" or "buff pink," the center in old caps reaching "terra cotta" or "cameo brown," or fading to the color of the margin, rarely in young fresh specimens

reaching a color as intense as "carnelian red" or "carrot red" or
"light coral red"; margin at first incurved; context white or tinged
very slightly with the color of the surface at least near the pellicle;
negative with $FeSO_4$ in the main part of the context of the pileus
and stipe (probably as in *G. glutinosus*); odor none; taste mild;
lamellae decurrent, rather broad (3–8 mm.) at least in medium and
large caps, close to subdistant, some forked near the margin and/
or near the stipe, whitish to "pale mouse gray" or "pallid neutral
gray," then "smoke gray" or "avellaneous" to "wood brown" and
eventually speckled darker by the spores which are between "bone
brown" and black in a good print; spores $12–22 \times 4.8–6.8\,\mu$,
mostly $14.2–18.5 \times 5.8–6.8\,\mu$ when taken from a print, subfusoid-
subcylindric, melleous-pallid to grayish brown or gray; basidia
$46–63 \times 10–12.5\,\mu$, 4-spored, very rarely very few 2-spored; cys-
tidia $65–145 \times 9–22\,\mu$, numerous, cylindric or somewhat ventri-
cose, rarely clavate, hyaline with some pale melleous to brownish
melleous granular resinous incrustations, thin-walled; subhymenium
and trama as in *G. glutinosus;* stipe tapering downward or sub-
equal, curved or straight, glabrous or very slightly fibrillose, white
except for the basal 20 or more mm.'s which are "empire yellow,"
"citron yellow," "amber yellow," "light cadmium" or "apricot yel-
low," the whole stipe tending to assume black spots, solid, $40–90 \times$
$5–20$ mm., veil sometimes forming a viscid, cortinoid but extremely
narrow easily gelatinizing annulus from the portion of the veil that
joins with the margin of the pileus, otherwise entirely glutinous
and hyaline, forming a sheathing covering up to a line close to the
very apex of the stipe, which is subpruinose, and unless this slimy
coating is washed off by heavy rains the surface remains shiny in
dried material; context concolorous with the surface; mycelium
often forming a grayish-melleous to grayish stramineous or (away
from the yellow base) more pallid grayish-fuscous mycelial tomen-
tum; all hyphae without clamp connections.

Type locality: Mt. Hood, Ore.; University of Michigan Herb.
(type seen).

Habitat: On humus and among mosses in coniferous woods and
open barrens, probably mostly associated with *Abies, e.g., A.
grandis,* possibly also with *Picea, e.g., P. Engelmannii,* or *Pinus,
e.g., P. ponderosa,* or *Pseudotsuga taxifolia;* fruiting from June
to January (the farther south the later).

Distribution: From British Columbia to Northern California and
to Montana and Colorado.

Illustration: Kauffman, Mycologia 17, pl. 13. 1925.

Var. *homobasis* Singer, Pap. Mich. Acad. Sci. **32**: 150.   1948.
Base of the stipe little or not colored yellow.   With the type.

12.   Gomphidius glutinosus (Schaeff. ex Fr.) Fries
Epicr. Syst. Myc., p. 319.   1838

Agaricus glutinosus Schaeff. ex. Fr., Syst. Myco. **1**: 315.   1821.
Cortinaria viscida S. F. Gray, Nat. Arr. Brit. Pl. **2**: 629.   1821.

Pileus fleshy, often very thin at margin, pulvinate to convex, then repand, becoming plane or somewhat depressed, obtuse or rarely subumbonate, 50–100 (137) mm.; surface glabrous, smooth, glutinous, shining when dry, "Rood's brown," "Verona brown," "Natal brown," "fawn color," then "walnut brown," "army brown," "pecan brown," or even "sorghum brown," occasionally reaching "carob brown," all these colors often pallescent or diluted with less pigmented spots, eventually often dotted by large "bone brown," or "fuscous" to black areas, reactions with $FeSO_4$, H_2SO_4 on pellicle negative, with NH_3 somewhat lilaceous pink; context white, becoming somewhat sordid in age, or assuming a very slight pinkish hue but unchanging when bruised, soft; odor none or almost none; taste mild or slightly acid; with NH_3 lilaceous pink, with NH_4OH sordid pink; formol in young specimens negative, in old ones slightly reddish after prolonged exposure; $FeSO_4$ negative; H_2SO_4 negative; the infrahypodermial zone with NH_3 distinctly purplish carmin to violet, with $FeSO_4$ greenish black to black, with H_2SO_4 pale brown, with formol quickly reddening; lamellae decurrent, forked, 5–10 mm. broad, subclose to distant, white then pale gray to "smoky gray" and eventually darker because of the spores; spore print between "bone brown" and black; spores 15–24 × 5–7.5 μ, mostly 15.3–20 × 5.2–6.2 μ in prints from American material, fuscous-gray or pale melleous-gray, cylindric-fusoid, with a slight suprahilar depression; basidia 45–55 × 9.5–12.5 μ, 4-spored; cystidia 90–160 × 12–18.5 μ, at first abundant, becoming scattered with age, hyaline or at times some of them with gray contents, subcylindric or somewhat ventricose with rounded apex; subhymenium filamentous or filamentous-cellular (if the septa are close enough), rather dense but looser than in the other sections, hyaline; hymenopodium rather dense, of interwoven hyphae; mediostratum pale melleous and rather dense, of axillar and subparallel hyphae; lateral stratum of subparallel hyphae, as a whole running parallel with the mediostratum, not diverging, looser than the mediostratum and the hymenopodium; stipe either subequal or tapering from the middle downward, often somewhat constricted at the apex, mostly straight, glabrous or very slightly longitudinally fibrillose, white or,

occasionally, becoming pale brownish, or with a very slight pinkish hue, except for the basal 25 or more mm. where it is "empire yellow," "wax yellow," or "primulin yellow," everywhere tending to assume large black spots (not glandular, however), solid, 40–90 × 10–22 mm.; veil sheathing the stipe with a slimy hyaline coating up to a circular annuliform line very close to the lamellae from where in young specimens the hyaline, entirely glutinous veil joins the margin of the pileus, consisting of hyaline, subparallel-subinterwoven, irregularly filamentous and non-incrusted hyphae of 0.8–4.2 μ diameter, imbedded in a glutinous mass; context concolorous with the surface but the white (upper) portion becoming sordid from the middle of the stipe outward, and then in the sordid portion reacting with $FeSO_4$ to greenish gray to black, otherwise reacting as in the pileus, the yellow (lower) portion however with $FeSO_4$ golden-yellow-subferruginous; all hyphae without clamp connections.

Type locality: Regensburg, Bavaria (no type specimens in existence).

Habitat: Among needles, grasses, mosses, in not too dense coniferous or mixed woods, the mycelium connected with mycorrhiza, preferably of spruce (*Picea* spp.) but also other coniferous trees (*Pinus*, possibly *Abies, Pseudotsuga, Tsuga*), fruiting from July to November, most commonly from August to October.

Distribution: Canada and United States (Northern States and some of the Southern States in mountainous regions), from the Atlantic to the Pacific Coast; also in Europe.

Illustrations: Bresadola, Icon. 671; Ricken, Blätterp. 2, pl. 3, fig. 1; Schaeffer, Bav. 4, pl. 36; Gramberg, pl. 9; Michael, pl. 32; Maublanc 1: 132 II; Cooke, Illustr. pl. 856 (879); Kauffman, Mycologia 17, pl. 12 (could also be *G. subroseus*); see also Saccardo, Syll. 19: 788.   1910.

Exsiccata: Herpell, Samml. praep. Hutp. 33, 89; Fuckel, Fung. Rhen. 1428; Petrak, Fl. Bon. Mor. 2164; Clements, Crypt. Form. Colo. 360; (*G. viscidus*); Saccardo, Mycol. Ital. 210.

Forma *maculosus* Quél. (ut var.), Flor. Mycol. p. 112.   1888. This is the white form (pileus white, not livid brown), occurring with the type form.

Note: The European and some of the eastern collections in this country have larger spores than the mid-western and western collections, which in many regards gradually become more and more
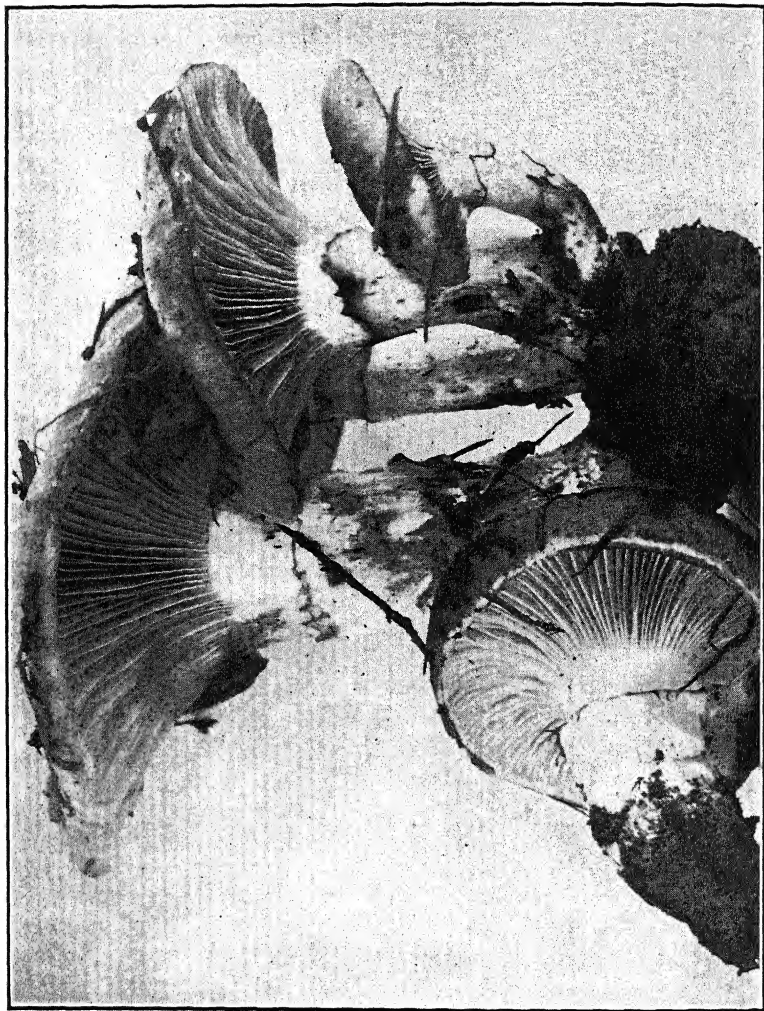
Photo A. H. Smith.

Fig. 3. *Gomphidius oregonensis.* × 1.

similar to *G. subroseus* as they enter the area of this species.   This intergradation may even occur occasionally in size and shape.   Consequently, it is not always easy to distinguish western collections of this species from *G. subroseus* without complete data on the fresh plant.

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Section *Microsporus* Singer

Papers Mich. Acad. Sci. Arts and Letters **32**: 149.   1948

13.   Gomphidius oregonensis Peck (Fig. 3)

Bull. Torr. Cl. **25**: 326.   1898

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Pileus fleshy, abruptly thin at margin, convex to expanded, becoming plane or slightly depressed, obtuse, 30–70 (100) mm.; surface glabrous, with a thick viscid to glutinous surface layer, "wood brown," "avellaneous," or with a flesh color hue, often black-spotted; margin incurved; context white, or with a slight pinkish hue; with KOH yellowish; with NH_4OH and NH_3 pinkish; with $FeSO_4$ instantaneously inky; odor none; taste mild; lamellae subdecurrent, close to subdistant, rather narrow, some or many forked, whitish becoming gray; spore print between "bone brown" and black; spores 11.5–14 (16) × 4.8–5.8 μ, melleous-gray to beautifully pure gray, cylindric to ellipsoid-subfusoid; basidia 40 × 9–10 μ, 4-spored; cystidia 90–125 × 8–12 (21) μ, thin-walled, with a fulvous-castaneous incrusting resinaceous hood, or with granular melleous incrustating fragments, hyaline to grayish, sometimes some with a purplish tinge in formal-KOH, cylindric or slightly ventricose, scattered to numerous; subhymenium filamentous, rather loose; hymenopodium dense and irregular, consisting of hyphae which project in all directions; mediostratum and the hymenopodium consisting of subparallel hyphae; stipe sometimes cespitose, subequal to subventricose, with tapering base curved or straight, glabrous to fibrillose, white above, bright lemon yellow below, tending to become blackish at places where handled or in age, or on drying, solid, (25) 60–110 × (5) 10–28 mm.; veil sheathing the stipe, like it does in *G. glutinosus,* up to the apical annuliform zone from which in young specimens a hyaline, glutinous veil extends to the margin of the pileus; mycelium often forming a tomentum at the base, bright yellow; context concolorous with the surface; all hyphae without clamp connections.

Type locality: Oregon, N. Y. State Mus. (type seen).

Habitat: Under *Pseudotsuga* and *Abies,* on the ground, fruiting from September to December.

Distribution: From Washington to California.

Note: Among the species of section Macrosporus this seems to be closest to *G. Smithii*. It grows plentifully and is edible as are all Gomphidii.

DOUBTFUL AND EXCLUDED SPECIES

Gomphidius roseus (Fr.) Karst. sensu aut. amer. The European species stands out because of its outwardly viscid but not entirely glutinous veil which would make it key out with *G. septentrionalis,* its constant association with *Pinus silvestris* and *Suillus bovinus* (L. ex Fr.) O. Kuntze, its rosy pileus ("light coral red" to "coral pink" at places reaching "carrot red," and margin near "pale flesh color" or "pale salmon color," or entirely in the latter colors), which makes it comparable with the American pink species, and the characteristic habit (short stipe, tapering downward to a slightly warm yellow or more often pink base). The spores are of the size found in section Macrosporus, viz. 17.3–24 × 5.8–7.5 μ; basidia 60–70 × 9–10.8 μ, 4-spored; cystidia 100–120 × 11.6–17.5 μ, rather numerous, little or not incrusted, cylindric or cylindric-ventricose, hyaline; subhymenium and trama as in *G. oregonensis;* chemical reactions similar to those of *G. glutinosus* but the base of the stipe reacting the same way as the infrahypodermial zone. The American authors mentioning *G. roseus* have not seen the real *G. roseus* but one of the American pink species, *i.e., G. septentrionalis, G. Smithii, G. nigricans, G. subroseus* (especially var. *homobasis*) and perhaps *G. oregonensis.*

Gomphidius stillatus Strauss in Sturm, Deutschl. Flora III, **33–34**: 3. 1853. The Bavarian type's position is somewhat doubtful though the European authors now generally agree that it is a form of *G. maculatus.* There is also a chance that it might be *G. glutinosus* f. *maculans.* *G. stillatus* has been cited by American authors as occurring in this country but here we have almost certainly either one of the above-mentioned forms or a young white stage of *G. nigricans.*

Gomphidius nigricans Peck sensu Atk., Mushrooms, p. 49. 1900. This is a stouter plant than the species described by Peck. We have not seen the latter vary as much as to include Atkinson's

conception as represented in his photographs although we do not exclude this possibility.

Gomphidius rhodoxanthus (Schw.) Sacc., Syll. **5**: 1139. 1887. This is not a *Gomphidius* but *Phylloporus rhodoxanthus* (Schw.) Bres. under which name it has been treated in North American Flora 10 (3); 193. 1917.

Gomphidius foliiporus Murr., Mycologia **35**: 432. 1943. This is not a *Gomphidius* but *Phylloporus rhodoxanthus* (Schw.) Bres. ssp. *foliiporus* (Murr.) Sing., Farlowia **2**: 280. 1945.

NOTES AND BRIEF ARTICLES

Three New Fleshy Fungi

Amanitopsis floridana sp. nov. Pileo plano, 5 cm. lato, avellaneo, striato, grato; lamellis liberis, albis, confertis; sporis ellipsoideis, levibus, 11–13 × 6–7 μ; stipite albo, glabro, clavato, 4 × 0.5–1.5 cm.; volva ampla, pallida, lobata, glabra, 2.5 × 2.5 cm.; volvella parva, alba, 5 mm. alta.

Pileus plane, 5 cm. broad, dull, glabrous, slightly viscid when wet, avellaneous with blackish disk, center smooth, margin closely striate for fully 1 cm., straight, fertile, entire, subconcolorous; context very thin, white, unchanging, odorless, mild; lamellae ventricose, crowded, inserted, milk-white, unchanging, free, abruptly depressed behind, about 7 mm. broad, entire; spores ellipsoid, smooth, uniguttulate, 11–13 × 6–7 μ; stipe inverted, peg-shaped, smooth, glabrous, white, unchanging, 4 × 0.5 − 1.5 cm., outer volva tough, not friable, ample, dirty-white, with three broad, acute lobes, glabrous, white inside, 2.5 × 2.5 cm.; inner volva (volvella) thin, white, about 5 mm. high, slightly lobed, closely encircling the base of the stipe like a collar.

Type collected by W. A. Murrill in rich soil at the base of a loblolly pine in a grove of frondose trees in Gainesville, Fla., August 5, 1948 (*F 21484*). This species looks like a combination of two, the cap greatly resembling that of *A. vaginata* and the volva suggesting that of *A. volvata* or *Amanita porphyria*. The spores, however, are not globose as in *A. vaginata* but ellipsoid like those of *A. strangulata,* which has a friable volva colored mouse-gray on the inside instead of white. To complicate matters still further, it is the tawny form of *A. vaginata,* not the gray, that has the secondary volva, a feature known also in *Amanita Caesarea* but not figured by Bresadola in his plate of that species. In my nomenclature this interesting addition to our flora would be *Vaginata floridana.*

Volvaria flaviceps sp. nov. Pileo campanulato, 3.5 cm. lato, flavo, fibrilloso; lamellis liberis, latis, albis, sterilibus; stipite albo, glabro, 4 × 0.6–1 cm.; volva ampla, 4 × 2.5 cm., subumbrina, squamosa.

Pileus campanulate, solitary, 3.5 cm. broad; surface dry, smooth, fibrillose, uniformly bright-flavous, margin fimbriate, projecting 2

mm.; context very thin, white, unchanging, with a peculiar sickening odor during drying; lamellae free, broad, wide and rounded at the margin, narrow behind, crowded, very thin, entire, white, unchanging, sterile; stipe smooth, white, glabrous, curved, enlarging below, 4×0.6–1 cm. above the volva, which is 4 cm. high and 2.5 cm. broad at the top, tapering to 1 cm. at the base, dry, dirty-white with large, flat, pale-umbrinous scales, their acute tips pointed upward.

Type collected by W. A. Murrill on the underside of a shaded magnolia log in Gainesville, Fla., July 11, 1948 (*F 22458*). A very rare, noteworthy, and beautiful species closely resembling *V. bombycina* in form and habit but with bright-yellow cap and scaly volva.

Boletus flocculosipes sp. nov. Pileo convexo-expanso, 9–14 cm. lato, fuligineo, imbricato-floccoso, grato; tubulis 1 cm. longis, 1–2 per mm., flavis, olivascentibus; sporis ellipsoideis, striatis, olivaceis, 12–14×6–7μ; stipite floccoso, rubro-brunneo, 6×2–2.5 cm.

Pileus convex to expanded, gregarious, 9–14 cm. broad; surface coarsely shaggy, fuliginous, margin concolorous, entire, fertile, incurved when young, with no trace of a veil; context pale-yellow, pinkish near the surface, bright-blue at once when cut, 1–2 cm. thick, odorless, mild; hymenium plane to somewhat ventricose, not depressed at the stipe, sulphur-yellow to dirty-sulphur and finally brownish, dark-blue-green at once when bruised; tubes 1 cm. long, 1–2 per mm., angular, not stuffed, inside same as mouths in color and change of color, taste mild; spores suggesting a long watermelon, oblong-ellipsoid, deep-olive-green when fresh, conspicuously longitudinally striate, uniguttulate, 12–14×6–7μ; stipe subequal, solid, 6×2–2.5 cm., hispid-shaggy, reddish-brown, yellow and glabrous at the apex, fuliginous at the base, bright-yellow inside, turning greenish-blue at once when cut.

Type collected by W. A. Murrill in bare soil near *Carya magnifloridana* Murr. in Gainesville, Fla., Aug. 7, 1948 (*F 39840*). Strongly suggesting *Strobilomyces* in the covering of cap and stem but not otherwise. In some ways it is rather close to *Frostiella* but that does not seem to be the natural place for it.—W. A. MURRILL.
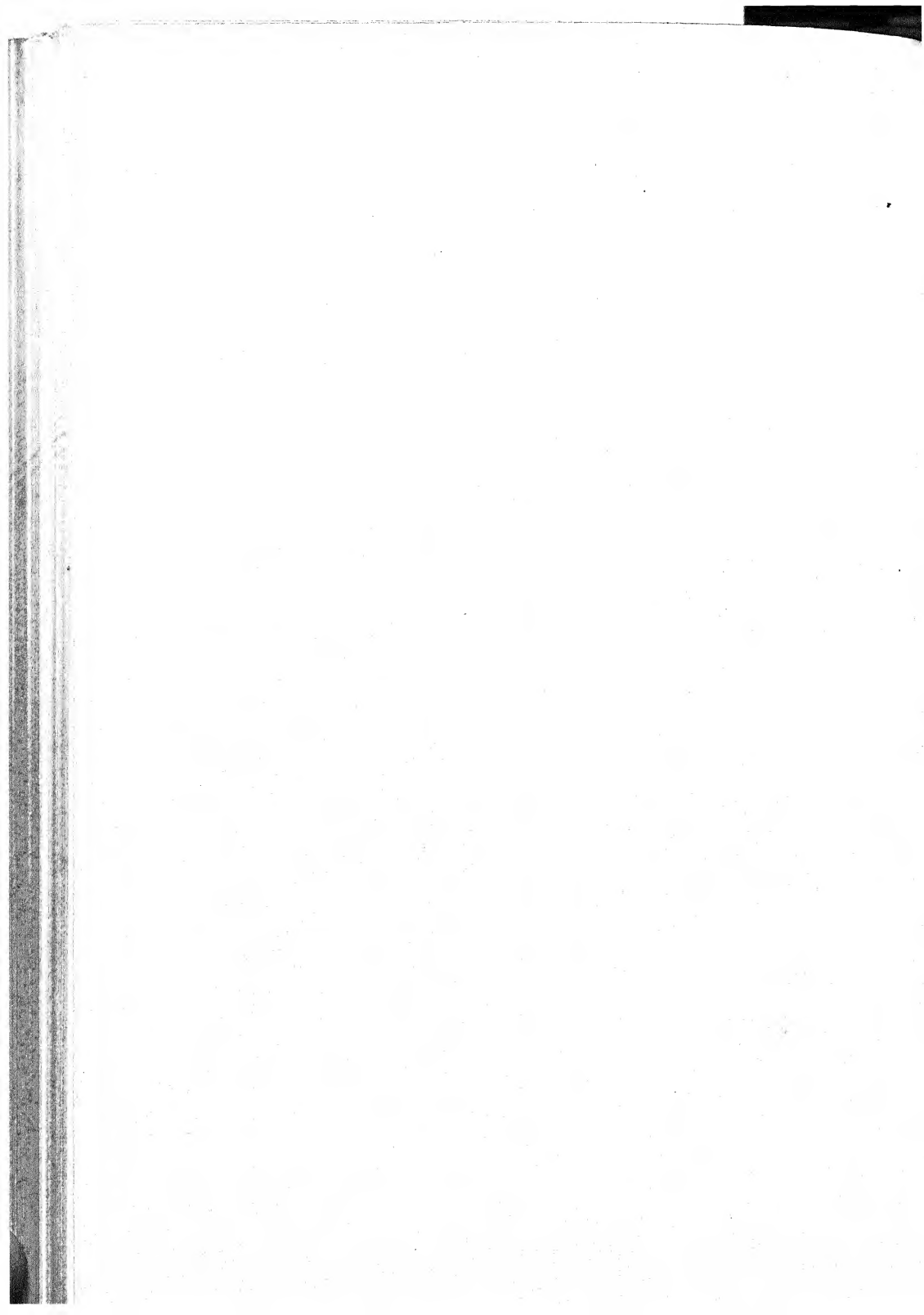
SOME LEAFSPOT FUNGI ON WESTERN GRAMINEAE—IV [1]

RODERICK SPRAGUE [2]

(WITH 1 FIGURE)

A study of additional collections of leafspot fungi on Gramineae, made in the interim since the last paper in this series was published, has disclosed the presence of additional noteworthy species in the mountains and plains of the west.[3] The recent seasons have been exceptionally favorable for the development of leaf fungi on Gramineae in Washington and adjacent areas. The precipitation from Sept. 1, 1947 to May 26, 1948 at Pullman, Wash., was 27.46 inches, compared with the 1893–1944 average of 17.94 inches for the period. The April precipitation was an inch over average and uniformly spaced, while that for May was 4.45 inches compared with an average of 1.47 inches for the month. The result of the prolonged, cool, overcast period with almost unbroken high humidity was an outbreak of leafspots unequaled in a quarter of a century. *Septoria tritici* Rob. in Desm. is an example. This species, which was found in considerable abundance in eastern Washington in June 1948, had not been collected east of the Cascade Mts. since 1915, even though it had been carefully searched for in other years.

[1] Published as Scientific Paper No. 831, Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman, Wash.

[2] Pathologist, Tree Fruit Exp. Station, Wenatchee, Wash.

[3] Sprague, R. Some leafspot fungi on western Gramineae—III. Mycologia **40**: 295–313. 1948.

[MYCOLOGIA for July–August (**41**: 357–491) was issued July 26, 1949]

Gloeosporium meinersii sp. nov.

Maculis foliicolis elongatis, 0.5–2.0 × 0.1–0.3 cm., fulvis v. ochraceis, emarginatis v. margine angusto, brunneo, centro ultimo pallido; conidiis numerosis, hyalinis, subphaseoliformibus v. oblongis, guttulatis, 9–12.5 × 3.0–3.8 μ.

Hab. in foliis vivis *Phlei pratensis* L., South Prairie, Pierce Co., Wash. July 8, 1948. Legit. Jack P. Meiners et George W. Fischer. **Typus** est **C.S. 3985.**[4] (Wash. State Coll. Dept. of Plant Pathology Herbarium.)

Spots on leaves elongate, 0.5–2 × 0.1–0.3 cm., pale buff to tawny, emarginate or with a narrow brown border, center of spots finally paler; conidia numerous, hyaline, non-septate, subphaseoliform to oblong, guttulate, 9–12.5 × 3.0–3.8 μ.

This fungus, occurring on living leaves, was compared with the conidial stage of *Phialea temulenta* Prill. and Del., the microspores of *Fusarium nivale* (Fr.) Ces., the conidia of *Gloeosporium bolleyi* Sprague and those of *G. graminum* Rostr. It matches none of these. It appears to be distinct and is worthy of recognition. Meiners found the fungus in his routine examination of collections made by him and G. W. Fischer in a grassy, drained-swamp region near Puyallup, Wash.

In addition to the type specimen I found a similar fungus on *Poa alpina* L. which Meiners, Fischer and I had collected on an exposed slope at 11,700 ft. elevation near Monarch Pass, Colo. (C.S. 17,102). The tawny lesions occupied much of the surface of basal leaves. The spores were hyaline, mostly non-septate, curved, subphaseoliform, 10–14 × 3–4 μ. This appears to be only a variant of *G. meinersii* for which the following name is proposed:

Gloeosporium meinersii var. **alpina** var. nov.

Maculis fulvis, emarginatis, acervulis obscuribus, sporulis curvuli-subphaseoliformibus, aseptatis, hyalinis, 10–14 × 3–4 μ.

Hab. in foliis sub-emortuis, *Poae alpinae* L., prope Monarch Pass, Colo. **Typus** est **C.S. 17,102.** Legit. Meiners, Fischer et Sprague, Aug. 7, 1948.

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[4] All C.S. numbers refer to the collection series of the Plant Pathology Department of the State College of Washington.

Spermospora subulata (Sprague) Sprague. What was formerly believed to be a relatively rare leafspot was found in abundance on *Melica spectabilis* Scribn. at Togwatee Pass, Wyo. It was, at least locally, very destructive, causing a blight of the entire plant. The brown, blasted plants were readily broken off at the ground line, leaving the bulbous culm-base in the soil. Many of the spores in this material were without a distinct, elongated apical appendage. However, some of the spores were typical of the fungus as originally described on *Melica subulata*.[5] The area where the diseased host occurred was a sub-alpine, semiforested meadow or prairie where the *Melica* grew in open spaces.

Linear, light brown to paler spots on leaves of *Agrostis alba* L., collected by Fischer, Meiners and Sprague at Teton Pass, Wyo. (C.S. 20,123), had spores typical for *S. subulata* except that an obliquely attached basal cilium about $10 \times 0.4 \mu$ was found on approximately 35 per cent of the spores in one mount. Spores of a second mount from the same collection, however, were virtually devoid of cilia. On referring back through all other available collections we found no cilia in material on *Melica* spp., nor on *Deschampsia caespitosa*, and one or two spores with cilia in material collected on *Calamagrostis*. We cannot find any difference in these various collections except in the occasional presence of cilia in some of them. While this is somewhat startling we are not segregating the two forms, ciliate and aciliate, except to the minor extent of emending the description of *Spermospora* to permit inclusion of the ciliate forms, and to present a formal description of the ciliate group as a morphological form of *S. subulata*.

Spermospora Sprague emend.

Conidia subulate to subulate-filiform, hyaline, septate, apical cell appearing appendage-like, sometimes with an oblique-basal cilium, borne superficially on evident short conidiophores in spots. A member of the Moniliales and the Moniliaceae.

Spermospora subulata f. **ciliata** f. nov.

Item ut in specie sed basibus sporulis ex obliquo ciliati, hyalini $10 \times 0.4 \mu$.

[5] Sprague, R. Undescribed species of *Cercosporella* and *Cercospora* on certain grasses in Oregon and Washington. Mycologia **29** (2): 199–206. 1937.

- Hab. in foliis vivis *Agrostis albae* (**type** C.S. **20,123**), Teton Pass, Wyo. et *Calamagrostis rubescentis,* Andrews Creek, Wash.

Same as species but with some of the spores carrying an obliquely attached basal cilium, $10 \times 0.4 \mu$.

The collection on *Calamagrostis* was reported recently with spores 50–80×7.0–8.4μ [6] but illustrated with spores 40–51×4.5–5.0μ taken from a second mount. The first measurements were taken from material which had been carried in a fishing basket for several days and apparently some of the spores were abnormally swollen. Probably the measurements 35–50×4.5–5.4μ, taken from another mount of this material, are closer to the typical condition.

OVULARIA PUSILLA (Ung.) Sacc. and D. Sacc. on *Festuca idahoensis* Elmer. A confusing leaf spot of Idaho fescue found in Idaho Co., Idaho, was eventually determined as being caused by *O. pusilla*. The black to purple-black lesions encircle the filiform or involute leaves and extend along the culm for a distance of 0.5 to 1.5 cm. While the lesions are numerous and relatively prominent, the hyaline conidiophores are difficult to find except on older lesions which have faded to an ashy hue. These older lesions are often bordered by wine-colored tissue. The spots were abundant on Idaho fescue growing along slopes above the South Fork of the Clearwater River, Idaho. This spot has been found on this host elsewhere in the Pacific Northwest but earlier collections were not identified because the fungus was either overlooked or the few spores that were present were not recognized. Very often only a few oval, hyaline spores were found in a mount and sometimes none.[7]

RHYNCHOSPORIUM ORTHOSPORUM Caldwell on *Dactylis glomerata* L. from Kendrick, Ida. (C.S. 17,014) differed from other collections on this host in the presence of 2- to 3-septate spores which were as large as $31 \times 4.0 \mu$. These spores were otherwise typical and were borne in characteristic white lesions. *R. orthosporum* was common on *Dactylis* in northern Idaho in 1948.

ERYSIPHE GRAMINIS DC. on *Calamagrostis rubescens* Buckl. The conidial stage of this common mildew was found in small

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[6] Loc cit., p. 308.

[7] Loc cit.; for a discussion of *O. pusilla* see pp. 309–311 of the 1948 paper.

quantities on leaves of pine grass below Hebgen Dam, Madison River, Mont. (C.S. 17,170). We have no record of mildew on *Calamagrostis* in the western area and this report may be of interest to those studying racial development in the species.

HELMINTHOSPORIUM VAGANS Drechsl. on *Poa bulbosa* L. The leaves, sheaths and vegetatively propagated heads of this grass were severely attacked at Pullman, Wash., during wet weather. This fungus, as usual, was common and destructive on *Poa pratensis,* but bulbous bluegrass has not been found infected with the fungus before. The fungus was identical with *H. vagans.* Probably the unusually wet season was instrumental in permitting attack of *P. bulbosa,* generally considered resistant in the field.

Phyllosticta minutaspora sp. nov.

Foliis dilute aureis, pycnidiis in lineis nigris dispositis, gregariis, substromaticis, pycnidiis minutis, carbonaceis, nigris, ostiolatis, globosis, 40–80 μ diam. Pycnosporulis minutis, hyalinis, bacillaribus, 3–4 × 0.5–0.7 μ.
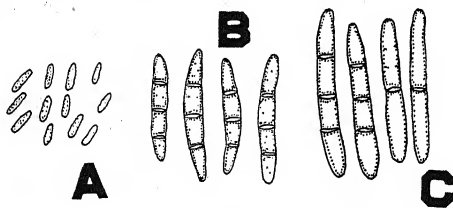
FIG. 1. *A,* pycnospores (microspores) of *Phyllosticta minutaspora* from type; *B,* pycnospores of *Hendersonia culmicola* var. *minor* on *Festuca rubra,* Newport, Oreg.; *C,* pycnospores of *H. culmicola* var. *minor* on *F. idahoensis,* Feather River, Calif. (All × 1,000.)

Hab. in foliis languidis *Muhlenbergiae filiformi* (Thurb.) Rydb. Prope Warm River, Ida. Legit. R. Sprague, Jack P. Meiners, et G. W. Fischer. Aug. 13, 1948. **Typus** est **C.S. 20,033.**

Infected leaves pale yellow, dying, pycnidia in black narrow lines, gregarious, somewhat or thinly surrounded by stromatic tissue, small, black, irregularly ostiolate, carbonaceous, globose, 40–80 μ diam. Pycnospores bacteria-like, hyaline, 3–4 × 0.5–0.7 μ (microspores).

On dying leaves of *Muhlenbergia filiformis* 10 miles north of Warm River, Idaho. Sprague, Meiners and Fischer Coll., C.S. 20,033 (type).

This species appears to be more parasitic than most *Phoma*-like fungi on Gramineae. The lower leaves of current season foliage turn yellow after invasion. The pycnidia are somewhat imbedded in fungus tissue, which gives a black appearance to the lesions. However, this tissue is interrupted and more or less *Phoma*-like. The fungus is related to a number of *Phoma* and *Phyllosticta* spp. with microspores (FIG. 1, *A*) but warrants recognition because of the very small spores. Its parasitic habit distinguishes it from the numerous saprophytic forms which are more or less morphologically similar. It would appear inadvisable to assign this fungus to one of the saprophytic types.

SEPTORIA AVENAE Frank on *Agrostis exarata* Trin. This blotch was collected by Meiners and Fischer in northwest Oregon near Elsie (C.S. 20,035). It showed globose, light brown pycnidia as much as 140 μ diam., containing 3-septate, cylindrical spores 33–39 $\times$ 3.2–3.8 μ.

SEPTORIA CALAMAGROSTIDIS (Lib.) Sacc. on *Agrostis rossae* Vasey was found at 11,000 ft. elevation in the Medicine Bow Natl. For., Wyo. (C.S. 17,037). The pycnidia were found on the lower leaves of this obscure host and contained narrow, curved, filiform spores ranging up to 48 μ long. The fungus is readily assignable to *S. calamagrostidis*.

SEPTORIA JACULELLA Sprague on *Bromus carinatus* Hook. and Arn. was collected at three places near Mesa Lake (Skyway), Colo. This represents a considerable extension of the range of this species.[8] It has now been found from Montana (on *B. ciliatus*) along the Madison River through Wyoming and Colorado south to Arizona and is, of course, abundant in the Pacific Northwest. The material from Colorado carried so many of the prominent black pycnidia that the leaves resembled those covered with the telial stage of a leaf rust. The spores were not as stiffly javelin-like as those from Washington and Oregon but they were accompanied by quantities of the bacteria-like microspores. The macrospores were stiffly curved to straight, 2- to 3-septate, 60–72 $\times$ 2.3–3.4 μ, mostly about 65 $\times$ 3.0 μ.

[8] Sprague, R. Septoria disease of Gramineae in Western United States. Oregon State Monographs, Studies in Botany No. 6. 1944 (Published by the Oregon State College, Corvallis). 151 pp.

SEPTORIA OUDEMANSII Sacc. on *Poa reflexa* Vasey and Scribn. The leaf sheaths of sub-alpine material of this host collected near Mesa Lake, Colo. (C.S. 17,071) carry a number of pycnidia containing spores comparable to those of *S. oudemansii*. The fungus differs somewhat from the common form on lawn grasses. The pycnidia are light colored with small pore-like ostioles which are surrounded by much darker cells than the rest of the cells of the pycnidia. The spores are also narrow for this species, many of them averaging about $17 \times 1.6 \mu$. They range up to 2.5μ diam., however, and this collection is therefore assigned to *S. oudemansii*. Material on *Poa pratensis* from the same area had spores 16–28 $\times 2.0$–3.0μ borne in pale, red-bordered spots. This approached *Septoria nodorum*. Material on *P. palustris* L. from Skyway, Colo., had spores 15–21.3×2.2–3.0μ.

SEPTORIA SECALIS var. STIPAE Sprague. Jack Meiners collected this fungus on *Stipa columbiana* var. *nelsonii* (Scribn.) Hitchc. along the South Fork of the Palouse River about seven miles up stream from Colfax, Wash. (C.S. 20,022). It is interesting to note that this variety, first described on *Stipa viridula* in South Dakota,[9] occurs a great distance westward from the type area. The Palouse collection, however, has the same cylindrical, hyaline spores as the type. They average 30–48×2.5–3.4μ, mostly 35–$45 \times 3 \mu$. After comparing critically with *S. avenae* and *S. avenae* f. sp. *triticea* T. Johnson, the writer is still satisfied that *S. secalis* is distinct because of its relatively narrower, more bacillar spores. Perhaps our variety is unnecessary but we maintain it for the reasons given at the time it was described.[10] The Palouse River collection is the first report of this fungus from Washington, but we have one collection on *Agrostis hallii* Vasey from Helmick Park, Oregon. The Palouse material is represented by scattered pycnidia in bleached areas and is usually associated with *Scolecotrichum graminis* Fckl.

HENDERSONIA CULMICOLA var. MINOR Sacc. on *Poa*. This fungus is a common saprophyte on dead grass leaves in the western United States. We have collected it many times. Spores of

[9] Sprague, R. Some leaf spot fungi on western Gramineae. Mycologia **33** (6): 655–665. Nov.–Dec. 1941.
[10] Loc. cit.

two collections on *Poa* spp. are, perhaps, somewhat distinct from the narrowly fusiform, 1- to 3-septate yellow spores found on *Festuca* (FIG. 1, *B*) and other hosts. One collection on *Poa gracillima* Vasey from Logan Canyon, Utah, made in 1947 (C.S. 3644), had cylindrical to subfusiform, yellowish, guttulate, 1-septate spores $16.5–19 \times 2.2–2.7 \mu$ borne in golden brown pycnidia 50–110 μ diam. Another collection on *P. secunda* Presl, from the South Fork of the Clearwater River, Idaho (C.S. 17,113), had sub-cylindrical spores $16–18 \times 2.5–3.4 \mu$. The spores were guttulate, then finally faintly 1-septate and yellow. These are similar to an earlier collection on *Festuca idahoensis* made along the Feather River in California (FIG. 1, *C*). Both collections had pycnidia which were scarcely ostiolate or tardily so. The material from Idaho gave some indications that the fungus was weakly parasitic as the pycnidia were in bleached to brown spots on dead parts of senescent leaves. The host in the Idaho material was taken from a rocky ledge which had, in recent years apparently, been overgrown with timber. Hence the habitat during this very wet year was abnormally unfavorable for this particular prairie species of *Poa*. We have seen little evidence that *H. culmicola* var. *minor* is actively parasitic. It appears to be a primary invader of dying leaf, culm or sheath tissue.

STAGONOSPORA SIMPLICIOR Sacc. and Berl. This saprophyte was found on basal leaves of *Festuca ovina* var. *brachyphylla* (Schult.) Piper at 10,000 feet elev. near Skyway, Colo. (C.S. 17,069). The lesions were obscure, and the dark brown pycnidia were relatively numerous and scattered or grouped along the filiform leaves. The ostiolate pycnidia were approximately 100 μ in diameter. The spores were mostly 1-septate, coarsely cylindrical, somewhat bent and resembled *Ascochyta utahensis* Sprague when young. Older spores were becoming 3-septate and the contents were coarse and faintly tinted. The spores measured $28–35 \times 7.4–10.0 \mu$ and, in this material, are not distinguishable from *St. simplicior*. The species seems to be an early invader of frost-injured tissue but it is scarcely parasitic.

STAGONOSPORA FOLIICOLA (Bres.) Bub. on *Glyceria*. A few prominent buff spots with a strong brown border were collected on *Glyceria elata* (Nash) Hitchc. ten miles north of Glendevey,

Colo., in a marsh (C.S. 17,121) on Aug. 9, 1948, by the writer and Jack P. Meiners. The pycnidia were deeply sunken, finally ostiolate, globose, gold-brown, 113–160 μ, scattered in the spots. The spores were blunt at the base, jointed at the apex and typically curved from being crowded in pycnidia which were none too large for the spores. The spores were hyaline, mostly 7-septate, 66–91 × 5.3–6.6 μ. The fungus is indistinguishable from *St. foliicola* except for the prominent border of the leaf spot. It is assigned to *St. foliicola* which causes a common spot on an associated marsh grass, *Phalaris arundinacea* L.

STAGONOSPORA VEXATULA Sacc. on *Deschampsia*. An immature pycnidial form on necrotic leaves of *D. caespitosa* (L.) Beauv. was obtained in a ditch near Plummer, Idaho, by the writer and C. Gardner Shaw June 16, 1948 (C.S. 3969). The pycnidia were slightly erumpent in lines in fuscous or stramineous spots, globose, flattened, ostiolate, parenchymatous, golden brown by transmitted light, 110–170 μ diam. The spores were hyaline, 30–40 × 4.5–5.5 μ, with numerous coarse oil drops and were 3-septate but evidently immature. They were cylindrical but somewhat pointed at the apex and slightly rounded at the base, giving them a slightly fusiform aspect in some cases. These spores seem to answer the description of *St. vexatula* Sacc. which occurs on *Phragmites communis* Trin. The pycnidia are much larger on *Phragmites* but it is believed that the ample food available in the culms and sheaths of *Phragmites* can account for this difference. *St. vexatula* is an early invader in necrotic leaves of the giant reed and it is probably only dubiously parasitic on *Deschampsia*. Since it shows some morphological similarities to the parasitic species *St. foliicola* (Bres.) Bubak it may warrant further observation as it may in some cases prove parasitic under the favorably humid habitat of *Deschampsia*. The harsh wiry leaves of this host are, however, in general, very resistant to invasion by leaf-spotting fungi.

STAGONOSPORA GRAMINELLA Sacc. Saprophytic material on *Agrostis oregonensis* Vasey from woods along the South Fork of the Clearwater River, Idaho, is assigned to this species. The flattened, globose, brown, ostiolate pycnidia are grouped on dead leaves. The nearly cylindrical, hyaline to faintly tinted spores measured 18–22 × 3–5 μ. The cells contained large guttulae.

This fungus is assigned to *St. graminella* but it was compared critically with *St. insularis* Speg., *St. agrostidis* Syd. and *St. smolandica* Eliasson, all of which occur on *Agrostis* and which have spores approximately the same dimensions as those of *St. graminella*. The comparative morphology of this group is as follows:

Name	Host	Pycnidium		Spore	
		Size, μ	Shape	Size, μ	Shape
St. agrostidis Sydow.	*Agrostis vulgaris*	180–250	globose-depressed	24 ×4	fusoid, curved to subfalcate
St. graminella Sacc. (Type)	grass	——	globose-papillate	18–20 ×3–3.5	cylindrical
St. graminella Sacc. (Local)	*Agrostis oregonensis*	80–145	globose-depressed	18–22 ×3–5	cylindrical
St. insularis Speg.	*Agrostis magellanica*	80–90	lenticular	18–24 ×3	cylindrical to subclavate
St. smolandica Eliasson	*Agrostis vulgaris*	80–90	globose-depressed	19–22 ×3	cylindrical

The only species which appear to match the Idaho material are *Stagonospora graminella* and *St. smolandica*. The latter is associated with a *Phyllachora* species and is ruled out in favor of the earlier named *St. graminella*.

STAGONOSPORA BROMI A. L. Sm. and Ramsb. A specimen collected on *Bromus carinatus* (C.S. 20,014) on the South Fork of the Clearwater River, Idaho, differs from other material in the spore dimensions. While most of the spores in this collection are short, 15–20 × 2.7–3.6 μ, some of them are as much as 42 × 4 μ. The spores are hyaline, cylindrical, less often subfusiform. The longer spores are sometimes blunt at the base and narrowed at the apical end. Therefore this collection ranges into *Septoria avenae, S. agropyrina,* or even *Stagonospora arenaria*. Under our present knowledge of this group it seems logical to retain *St. bromi* and not assign the fungus to *S. avenae*.

All of the collections of *Stagonospora bromi* have the black or purple-brown leafspots characteristic of the species. Whether this is really a specific character or is related to the reaction of bromes to parasitic fungi is open to question. Certainly most leafspots on bromes are dark brown or black.

Black stem on grasses. A number of collections were made of a black stem disease on various species of grasses in northern and west-central Idaho in 1948. The stem for several inches or sometimes its entire length was black as though it had been burned. In one specimen on *Festuca elatior* L. near Post Falls, Idaho, small subsuperficial black pycnidia were formed and the entire blackened area was invaded by very fine, dark, somewhat superficial mycelium. The pycnidia contained hyaline microspores 4–6 × 0.5 μ diam. These were spermatia-like and some of the pycnidia appeared to contain ascogenous initials. This specimen (C.S. 17,079) is filed in the herbarium of the Dept. of Plant Pathology, W.S.C., under *Phoma* sp. I see no reason for describing this species until detailed work is done with it. The same fungus was found on *Festuca idahoensis* Elmer at 10 miles north of Warm River, Idaho (C.S. 20,051), Targhee Pass, Idaho (C.S. 17,133), and in adjacent Mont. (C.S. 17,133). Probably the same fungus was involved in the same symptoms on *Festuca idahoensis* near Desmet, Idaho and on *F. occidentalis* Hook. near St. Regis, Mont. (C.S. 20,077). Material on *F. idahoensis* at McCall, Idaho (C.S. 20,013), was confined to the leaves which were jet black. No pycnidia were present but small mycelial aggregates up to 30 μ in diameter were scattered among the black mycelia. A few spores of *Cladosporium* sp. were present. Black streaks with numerous erumpent ostiolate pycnidia (30–60 μ) were noted on culms and sheaths of *Agropyron spicatum* (Pursh.) Scribn. and Sm. collected on the South Fork of the Clearwater River, Idaho (C.S. 20,016). The aseptate hyaline spores were bacillus-like, 3.5–5.5 × 0.4–0.6 μ. The writer and colleagues saw these symptoms on a number of other hosts but field examination failed to disclose any organism and frequently the material was discarded without further study. Similar material was collected by C. L. Lefebvre on *Festuca* spp. in Alaska.

The black-stem fungus produces a much more extensive charring than that caused by *Phyllosticta minutaspora*.

Scolecotrichum graminis Fckl. Abundant material on *Muhlenbergia filiformis* (Thrub.) Rydb. from McCall, Ida., another unreported host, brings the total number of hosts parasitized by this fungus to 114 species for the western United States, 132

for the entire country and about 160 species for the world. In
recent collections as in earlier ones, this fungus is one of the most
abundant and widespread species which we have dealt with. The
fungus was common in the mountains of Colorado and Wyoming
on *Bromus ciliatus* L., *B. frondosus* (Shear) Wootn. and Standl.,
B. purgans L., *Phleum alpinum* L., and a number of the well
known hosts including *Agropyron, Elymus* and *Poa* spp. The
fungus was exceptionally destructive on *Melica subulata* (Griseb.)
Scribn. and *Bromus carinatus* Hook. and Arn., among others, in
the Umatilla Natl. For., Wash., during June 1948 following pro-
longed rains. *Stipa lemmonii,* in the same area, was nearly killed
by the leafstreak fungus. While there is considerable variation in
size, especially length of spores, of this fungus on the different
hosts, all of the collections are readily assignable to the fungus
which we call *S. graminis.*

I am indebted to my colleagues, C. Gardner Shaw, Jack P.
Meiners, and George W. Fischer, for aid in collecting specimens
discussed in this paper.

NEW MONOCENTRIC EUCARPIC OPERCU-
LATE CHYTRIDS FROM MARYLAND [1]

JOHN S. KARLING

(WITH 78 FIGURES)

In addition to the polycentric chytrids previously reported by the author (**1949**) from Maryland, a large number of monocentric eucarpic species were collected and isolated from soil and water. Among these were six new endo- and exoöperculate species which are characterized by large sporangia with one to several exit papillae or tubes and coarse, richly-branched rhizoids which usually arise at several points on the periphery of the sporangium. Accordingly, they apparently belong in the genus *Karlingia* which includes *Rhizophlyctis*-like chytrids with operculate sporangia.

This genus was established by Miss Johanson (**1944**) for the long known and commonly occurring chytrid, *Rhizophlyctis rosea,* which she found to be endoöperculate. She, accordingly, removed it from *Rhizophlyctis* and renamed it *K. rosea.* Since that time the concepts of the genus have been extended to include exoöperculate species as well (Karling, **1947**). At present it includes four species, all of which were isolated and grown on cellulosic substrata and whose occurrence in nature appears to be limited to such natural media. Four of the Maryland species, on the other hand, were isolated on chitin and are distinctly chitinophilic. The other two new species from Maryland were isolated and grown on onion skin, grass leaves and cellophane, and could not be transferred to chitin.

All of these species differ in several respects from the known members of *Karlingia,* as will become evident in the diagnoses

which follow. They are, accordingly, regarded as new species and named *K. chitinophila, K. asterocysta, K. curvispinosa, K. dubia, K. lobata,* and *K. marylandia.*

In all of these species the sporangia and resting spores usually develop directly from the zoospore as has been described for the other members of the genus. However, in a low percentage of the thalli they may develop from a local enlargement of the germ tube, and in such cases the zoospore cyst may become thick-walled and persist as an appendage to the sporangium or resting spore. Also, rare polycentric thalli may occur in all species, as has already been reported for *K. granulata, K. hyalina* and *K. rosea* (Karling, 1947). Furthermore, in most thalli of the Maryland species numerous rhizoids are present and arise at several points on the periphery of the sporangium, but it is not uncommon to find sporangia with only one or two basal rhizoids.

Karlingia chitinophila sp. nov. (FIGS. 1–8)

Fungus saprophyticus. Sporangiis plerumque totaliter extramatricalibus, hyalinis, laevibus, sphaericis, 10–215 μ, ovalibus, 15–30 × 20–45 μ, pyriformibus, 32–50 × 60–100 μ, citriformibus, elongatis, vel angularis aut irregularibus, 1–26 papillis exeuntibus vel tubulis, 12–70 μ longitudine × 5–8 μ diametro ab infirmo, quarum fines impletae sunt obturamento hyalino glutinoso, quod usque at 5–15 μ supra verticem terminari potest. Rhizoidibus plerumque a pluribus locis in sporangiorum superficie emergentibus, principibus axibus plerumque crassis, diametrum, attingentibus 20 μ, maxime ramosis et extensis. Operculo hyalino, tenui, submerso in papillis exeuntibus vel tubulis, vadoso, figura ad modum patellae vel crateris usque ad 12 μ diametro, expulso et ablato, zoosporis emergentibus, aliquibus-operculis accessoriis desorptis in sporangia, zoosporis emergentibus et pressione interna relaxante. Zoosporis hyalinis, sphaericis, 3.18–3.71 μ, plurimis granulis minutis praestantibus, flagello 22–24 μ longitudine. Sporis perdurantibus levibus, sphaericis, 8–26 μ, ovalibus, 8–18 × 11–23 μ, oblongatis, angularibus vel irregularibus, pariete crasso praeditis, 2–6.3 μ diam., colore olivaceo, rebus contentis crasse granulosis. Germinatione a prosporangio.

Saprophytic on chitinous substrata in soil and water, Frederick and Keesville, Md.

As is evident from the above diagnosis, this species is almost the hyaline chitinophilic counterpart of *K. rosea.* Therefore, it need not be illustrated extensively for adequate description, and the accompanying drawings (FIGS. 1–8) are limited to the zoospores and resting spores. Except for its hyaline color, smaller
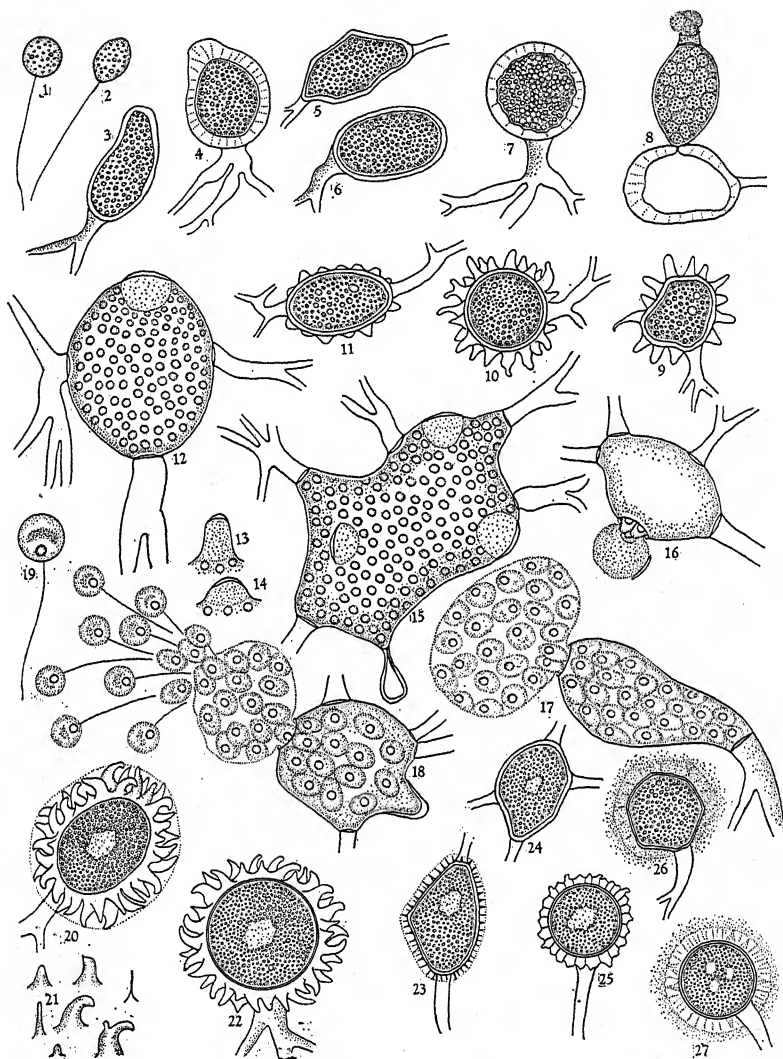
FIGS. 1–27. Species of *Karlingia*.

zoospores (FIGS. 1, 2), and liking for chitin it is identical to and might readily be mistaken for unpigmented specimens of *K. rosea*. However, when it is studied intensively distinctive differences become apparent. The zoospores are from 1 to $2\,\mu$ smaller in diameter, more nearly spherical while swimming, and have a

shorter flagellum. Also, the included granules are hyaline instead of faintly rose or pink colored. As a result the protoplasm in the sporangia is always hyaline to greyish granular in appearance. Thalli have been grown under a wide variety of temperature and light conditions, but the sporangia never varied in pigmentation, as often occurs in *K. rosea* under such conditions.

Another striking difference is the inability of *K. chitinophila* to grow on cellulosic substrata. Numerous attempts have been made to grow it on cellophane, onion skin, bleached grass segments, filter paper, cotton fibers, etc., without success. It has a distinct liking for purified chitin, and bits of this material may become completely overgrown in a few days. However, this species is not completely limited to growth on this type of substratum, but like many other chitinophilic species it may grow to a limited extent on dead keratinized tissues such as skin and hair.

The resting spores also differ somewhat from those of *K. rosea* and the other known members of the genus. They vary markedly in size and shape (FIGS. 3–8) and are greenish-brown in color. The wall may become exceptionally thick (FIGS. 4, 7, 8), and within it may occur faint radial lines or striations. Also, the inner periphery of the thick wall often appears to be irregular (FIG. 7). The resting spores developed very abundantly in the collections from Keesville and Frederick, Md. However, in a culture isolated from moist soil from Liberia, Africa,[2] no resting spores have developed in the course of fourteen months. Whether or not heterothallic strains occur in this species, as Couch (**1939**) reported for *K. rosea,* is not known, since no attempts have been made to cross the Liberian culture with the ones from Maryland.

The sporangia of *K. chitinophila* show the same degree of variation in size, number of exit papillae, position and number of rhizoids as those of *K. rosea, K. spinosa,* and *K. hyalina.* The small and medium-sized sporangia at the edges of the substratum, however, often develop long exit tubes instead of papillae. These tubes taper gradually towards the apex and may vary from 12 to 70 μ in length by 5 to 8 μ in diameter. During the late stages of development the tips of the exit papillae and tubes deliquesce,

after which plugs of hyaline material become visible in the orifices (FIG. 8). At the same time endoöpercula form beneath the plugs at varying depths in the papillae and tubes. Apparently, by this time cleavage of the protoplasm into zoospore initials has occurred because the sporangia appear to be filled with fairly well-defined segments. This phase, similar to that shown in figure 8, may persist from several hours to more than a day before dehiscence occurs. Similar persistence of what appears to be a post-cleavage phase has been observed in *K. asterocysta, K. curvispinosa* and *K. dubia,* and this seems to be a fairly distinct characteristic of these chitinophilic species.

During dehiscence the operculum is pushed up and out by the emerging mass of zoospores in the same manner described by Miss Johanson (**1944**) and the author (**1947**) for *K. rosea* and *K. hyalina.* This usually occurs so quickly that the operculum may be completely overlooked unless it is under direct observation at the moment of dehiscence.

Karlingia asterocysta sp. nov. (FIGS. 9–19)

Fungus saprophyticus. Sporangiis hyalinis, laevibus, sphaericis, 20–110 μ, ovalibus, 15–30 × 28–45 μ, pyriformibus, 12–40 × 29–75 μ, oblongatis, elongatis, fusiformibus, vel angularibus; 1–4 papillis exeuntibus, 8–14 μ diam. Rhizoidibus plerumque a pluribus locis in sporangiorum superficie emergentibus, principibus axibus plerumque crassis diametrum attingentibus 18 μ maxime ramosis et extensis. Operculo hyalino, vadoso, figura ad modum patellae vel crateris usque ad 14 μ diam. Zoosporis hyalinis, sphaericis, 4.2–4.6 μ, cum uno globulo refractivo hyalino, 0.7–1.2 μ diam., flagello 24–26 μ longo. Sporis perdurantibus sub sphaericis, 15–30 μ, ovalibus, 12–16 × 14–22 μ, oblongatis, angularibus vel irregularibus, spinosis vel verrucosis, colore olivaceofulvosis, contentis granulosis. Germinatione ignota.

Saprophytic on chitinous substrata in soil and water along routes 232 and 234, Charles County, Md.

This species, as noted above, is characterized primarily by large exoöperculate sporangia (FIGS. 12, 15) and somewhat stellate or asteroid resting spores. The latter are slightly similar to the resting spores of *K. spinosa* but otherwise the two species differ in several respects. *Karlingia asterocysta* lacks the golden-orange pigmentation of the former species, has slightly larger zoospores and exo- instead of endoöperculate sporangia, and a distinct predilection for chitin.

Resting spores occur very abundantly in this species, and in most subcultures studied they developed before and in greater numbers than the zoosporangia. As shown in figures 9 to 11 they are characterized by the presence of blunt to sharp-pointed, tapering, conical pegs which vary from 22 to 60 in number per spore and are 4 to 8 μ high by 2 to 4 μ broad at the base. Occasionally, the tips of the pegs may be slightly curved (FIG. 10). In two spores observed the pegs were reduced to warts with the result that the outer wall was only verrucose. At maturity the pegs and inner wall are usually dark greenish-brown in color, while the content of the spore is hyaline and finely granular (FIGS. 9–11).

In addition to its characteristic resting spores, *K. asterocysta* may be distinguished usually from the other chitinophilic species by its low, inconspicuous and barely perceptible exit papillae, which seldom project much above the surface of the sporangium (FIGS. 12, 15). Nevertheless, their position can be readily detected by the presence of a large hemispherical area of hyaline matrix underneath. In large sporangia these areas may be as much as 15 μ across and extend to a depth of 16 μ in the sporangium. It must be noted, however, that in small sporangia the exit papillae may become fairly conspicuous and up to 11 μ high (FIGS. 13, 14). Therefore, the size of the exit papillae is not always a reliable diagnostic character.

Another distinguishing characteristic of this species is the swarming of the zoospores for a brief period in a vesicle outside of the sporangium. So far, this has not been observed in the other chitinophilic species. During dehiscence the operculum is pushed up and the slimy matrix exudes first to form a globular mass at the exit orifice (FIG. 16). The emerging zoospores push up into this mass and expand it, until it finally forms only a thin layer around the zoospores as in species of *Chytriomyces* (Karling, 1947B), *Asterophlyctis* (Antikajian, 1949), and other chytrid genera. Within a few seconds to 1.5 minutes the zoospores begin to jerk and move about in the mass, and shortly thereafter they start swarming actively in a confined and localized area. This area is bounded by a hyaline, thin, vesicular membrane, which apparently originated from the matrix surrounding the emerging

zoospores (FIG. 17). They swarm in this vesicle briefly for 50 to 80 seconds, after which it ruptures and the zoospores escape (FIG. 18). In the case of large sporangia, however, the vesicle may rupture before most of the zoospores have been discharged, and its presence, accordingly, is of brief duration.

Karlingia curvispinosa sp. nov. (FIGS. 20–35)

Fungus saprophyticus. Sporangiis hyalinis, laevibus, sub-sphaericis, 10–120 μ, ovalibus, 10–80 × 15–140 μ, pyriformibus, 8–70 × 18–160 μ, obclavatis, oblongatis, aut elongatis; 1–3 papillis exeuntibus. Rhizoidibus plerumque a pluribus locis in sporangiorum superficie emergentibus, principibus axibus plerumque crassis diametro attingentibus 16 μ, maxime ramosis et extensis. Operculo apiculato in papillis exeuntibus, 6–18 μ diametro. Zoosporis hyalinis sphaericis, 3.8–4.2 μ, cum uno globulo refractivo hyalino, 0.6–0.8 μ diam.; flagello 10–13 μ longo. Sporis perdurantibus sphaericis, 6–21 μ, ovalibus, 8–12 × 11–16 μ, minime angularibus et irregularibus, spinosis vel verrucosis, rebus contentis granulosis; germinatione a prosporangio.

Saprophytic on chitinous substrata in soil and water from a ravine near the Monocacy River at Frederick, Md.

This species resembles *K. asterocysta* in having asteroid resting spores, but the pegs or spines are usually curved and somewhat hook-like and more numerous. In addition, the zoospores are markedly smaller and have a shorter flagellum than those of the previous species. Furthermore, they do not swarm in a vesicle outside of the sporangium. *K. curvispinosa* is also more prolific in growth, and bits of purified chitin usually become completely overgrown with sporangia and resting spores in the course of two weeks. Also, it develops unusually fast and may produce mature sporangia and resting spores in two days. The resting spores develop as abundantly and rapidly as the sporangia, and because of their color the chitinous substratum becomes greenish-amber in appearance within a few days.

Unlike the other known species of the genus, the young incipient resting spores are surrounded by a relatively clear zone of amorphous material in which faint radial lines or striations occur. The pegs or spines gradually develop in this layer as the spores mature and appear to be formed from its material (FIGS. 26–28) in much the same manner as Butler (1905) and McLarty (1942) described for *Olpidiopsis*. Even in mature spores the

FIGS. 28–51. Species of *Karlingia*.

outer boundary of the layer may sometimes persist, so that the spores appear to lie in a faintly-visible, delicate vesicle (FIGS. 20, 23). In longitudinal view the pegs appear to be pyramidal in shape, usually blunt and abruptly tapered, 4–7 μ high by 3–5 μ broad at the base, and curved or hooked at the tip (FIGS. 20–22).

Sometimes they may be so numerous and closely packed together that they appear to be fused and to form a radially striated outer wall (FIG. 25). Occasionally, the pegs may be reduced to low warts, so that the spores are distinctly verrucose. In rare cases the spores may be echinulate (FIG. 23) or even smooth (FIG. 24). Their content is evenly granular with a small irregular central vacuole, and at maturity the outer wall and pegs are dark-amber or brown in color. During germination they function as prosporangia and form superficial thin-walled zoosporangia. During this process and for several weeks afterwards the pegs persist as fairly rigid structures.

The sporangia vary markedly in size and shape (FIGS. 29–34) as in the previous species and have from 1 to 3 exit papillae which may be low and inconspicuous or somewhat cone- or dome-shaped and prominent (FIGS. 29–33). During dehiscence the operculum is pushed off, and disappears quickly so that its presence can be determined only at the moment of dehiscence. Sometimes the sporangia may be stalked (FIG. 33) and stand off from the substratum to a distance of 25 to 60 μ. Occasionally, ingrowing plugs of wall material occur in the sporangia as in species of *Chytriomyces* (Karling, 1947).

Karlingia dubia sp. nov. (FIGS. 36–51)

Fungus saprophyticus. Sporangiis laevibus, hyalinis, sub-sphaericis, 20–240 μ, ovalibus, 45–65 × 60–75 μ, pyriformibus, 15–35 × 40–78 μ, vel oblongatis, 1–4 papillis exeuntibus, 12–34 μ diam. Rhizoidibus plerumque a pluribus locis in sporangiorum superficie emergentibus, principibus axibus plerumque crassis diametro attingentibus 12 μ, maxime ramosis et extensis. Operculo apiculato, vadoso, figura ad modum patellae vel crateris usque ad 10–30 μ diametro. Zoosporis hyalinis, sphaericis, 6–6.5 μ, cum uno globulo refractivo hyalino, 2–2.3 μ diam.; flagello 32–35 μ longitudine. Sporis perdurantibus laevibus vel rugosis et verrucosis, fuscis, sphaericis, 8–20 μ, ovalibus, 9–14 × 12–17 μ, minime elongatis aut angularibus, rebus contentis granulosis. Germinatione ignota.

Saprophytic on chitinous substrata in soil and water, route 504, Calvert County, Md.

The primary characteristics which distinguish this species are its large exoöpercula and zoospores, and smooth to rugose or verrucose, brown resting spores. Its zoospores are considerably

larger than those of the previously described species, and the operculum has an unusual relation to the sporangium. At maturity it appears to be pushed up and separated from the sporangium wall and to rest on a hyaline, broadly conical cushion (FIGS. 36–38). Its appearance and position relative to the sporangium at this stage is somewhat similar to that in germinating resting sporangia of *Physoderma zeae-maydis* (Tisdale, **1919**). Sometimes, the distance between the lower edge of the operculum and the exit orifice may be as much as 3 to 5 μ, and the line of separation between the two structures may be jagged as well as smooth and even (FIGS. 37–39). When opercula and empty sporangia are examined under the oil immersion lens, however, an inner vesicular membrane or wall becomes visible, which projects out of the exit orifice for a short distance. This is particularly evident in surface and side views of the exit orifice, as shown in figure 39. Apparently, it is this inner vesicle or sporangium wall which grows out into the exit orifice and pushes the operculum up. However, it is not certain that this vesicular membrane is the inner layer of the sporangium wall as suggested in figures 36–40. On the other hand, it may be the wall of an endosporangium of the type present in the germinating resting sporangia of *Physoderma zeae-maydis*.

Underneath the raised operculum is an almost hemispherical area of hyaline matrix which may be 12 μ across and extend to a depth of 16 μ in the sporangium (FIGS. 36–38). This material escapes first as the sporangium dehisces and forms a large globule at the exit orifice (FIG. 40). The emerging zoospores slowly push up into this globule and expand it. At first the expanding mass of zoospores and surrounding matrix is almost spherical, but it soon becomes flattened at the base or even slightly invaginated (FIG. 41) as more zoospores push up into it. In a short while the zoospores begin to separate, and at this stage the flagellum is coiled around the body of the spore (FIG. 42). The flagellum soon uncoils and begins to beat, and within one to two minutes after emerging the zoospores swim away. The swimming zoospore is spherical in shape (FIG. 43) with a conspicuous hyaline refractive globule which usually lies near the base. If zoospores are trapped in a narrow space they may elongate, and in such

instances the refractive globule appears to lie in a vacuole (FIG. 44). In old sporangia cleavage may be very irregular so that large spherical, 8–20 μ, zoospores are formed which contain several refractive globules and have two to eight flagella.

Another noteworthy character of *K. dubia* is the thick-walled and irregularly constricted rhizoids (FIGS. 36, 40, 45, 46). This is particularly distinctive of old and large thalli. In this respect the rhizoids are somewhat similar to those of *K. granulata,* with the exception that the thickening is fairly uniform and may extend almost completely across the lumen of the rhizoid in the constricted regions. Constricted rhizoids, however, are not universal in this species, and in numerous thalli they may be fairly straight with few or no constrictions.

As noted in the diagnosis above, the resting spores are usually smooth (FIGS. 50, 51), but encrusted (FIG. 48), and slightly verrucose spores (FIG. 49) occur fairly commonly. Their content is coarsely but evenly granular with a central, slightly angular vacuole, and the wall is dark brown in color. So far germination of the resting spores has not been observed.

The other two new species, *K. lobata* and *K. Marylandia,* differ from the preceding ones in that they will not grow on chitinous substrata. So far they have been cultured only on onion skin, grass leaves, cellophane, and to a limited extent on dead human skin.

Karlingia lobata sp. nov. (FIGS. 52–69)

Sporangiis hyalinis, sphaericis, 15–180 μ, pyriformibus, 12–40 × 20–160 μ, ovalibus, 9–80 × 14–98 μ, oblongatis, elongatis vel irregularibus 1–4 papillis exeuntibus. Rhizoidibus plerumque a pluribus locis in sporangiorum superficie emergentibus, principibus axibus plerumque crassis diametro attingentibus 28 μ, maxime ramosis et extensis. Operculo hyalino, apiculato vel submerso in papillis exeuntibus, vadoso, figura ad modum patellae vel crateris usque ad 20 μ diametro. Zoosporis hyalinis, sphaericis, 5.2–6.1 μ, plurimis granulis minutis praestantibus, flagello 30–32 μ longo. Sporis perdurantibus hyalinis, laevibus, lobatis, irregularibus, angularibus, 7–38 μ diam., germinatione a prosporangio.

Saprophytic on cellulosic substrata in soil and water, route 504, Calvert County, Md.

This species is very similar to *K. granulata* by its endo- and exoöperculate sporangia, irregularly constricted, thick-walled rhi-
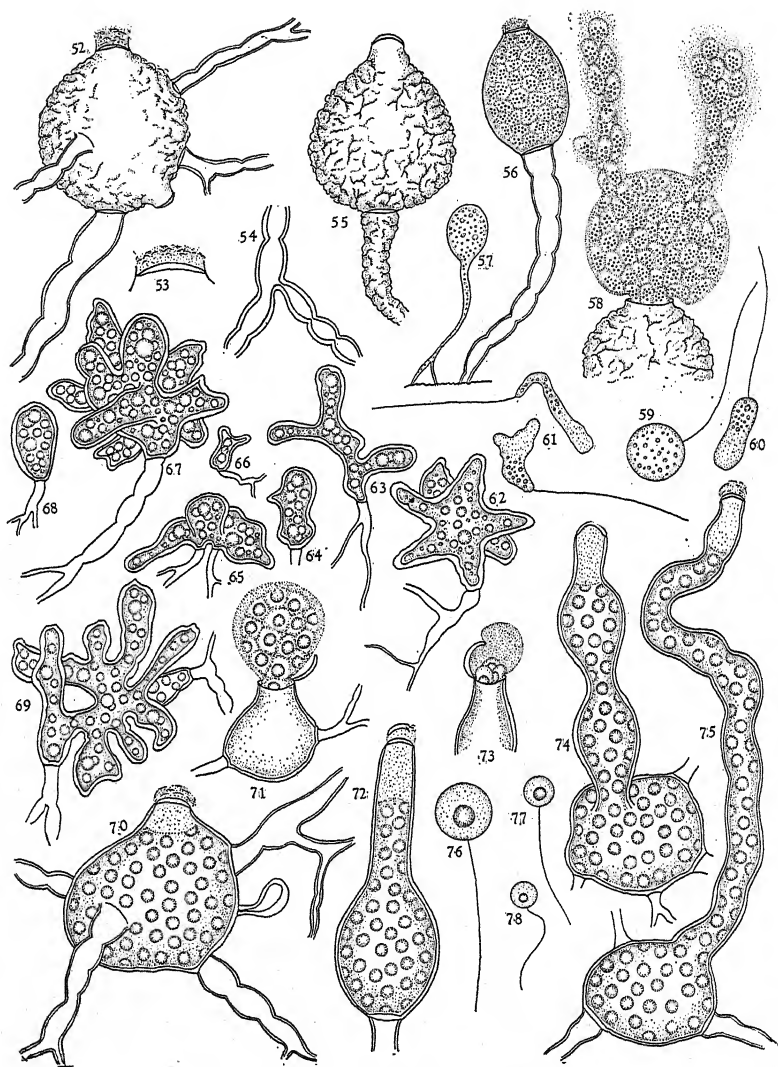
FIGS. 52–78. Species of *Karlingia*.

zoids, and zoospores which include from 8 to 20 hyaline refractive granules.   In fact the similarity is so striking that until the resting spores were found it was identified as *K. granulata*.   In view of this, extensive illustration of this species is not necessary for an adequate description of it and only the most striking differences will be drawn.

Instead of being spherical to oval in shape and brown in color as in *K. granulata,* the resting spores are hyaline, smooth and usually deeply-lobed or irregular in shape and contain numerous small hyaline refractive globules (FIGS. 62–69). In shape they resemble the resting spores of the polycentric chytrid, *Septochytrium plurilobulum* (Johanson, 1943). A few small oblong and only slightly irregular spores (FIGS. 64, 66, 68) have been found, but they appear to be exceptional. All spores begin as globular enlargements of the germinated zoospores, but the subsequent growth and enlargement is not uniform. As a result numerous protuberances and lobes develop, and eventually the irregular and deeply-lobed spores are formed. After maturity they germinate in the typical chytrid manner by forming a thin-walled zoosporangium.

Another characteristic which distinguishes the large and old sporangia of this species is the structure of their wall. It usually becomes quite thick and wrinkled or ridged, so that such sporangia have a distinctive appearance (FIGS. 52, 55, 58). Sometimes the wrinkling may extend to the basal portion of the rhizoids as well (FIG. 55). As noted earlier, the rhizoids are usually irregularly constricted (FIG. 54), fairly thick-walled, coil extensively in the surrounding water, and may extend for a distance of 1,500 μ. In exceptional monorhizoidal thalli, the main axis may be up to 28 μ in diameter.

The zoospores (FIGS. 59–61) are approximately the same size as those of *K. granulata* and usually vary from 5.2 to 6.2 μ in diameter. However, smaller, 3.9–4.2 μ, and larger, 6.5–7 μ, ones may be formed, but these appear to be abnormal. The refractive granules vary considerably in number and size, and some of them may be distinctly globular and up to 0.4 μ in diameter. While swimming the zoospores are spherical, but when they come to rest and creep about intermittently they become elongate and amoeboid (FIG. 61). The anterior end becomes lobose and relatively clear (FIGS. 60, 61), while the posterior end contains the granules. The zoospores germinate readily in water, particularly in the vicinity of the onion skin, and give rise to free growing thalli of which only the tips of the rhizoids penetrate the substratum (FIGS. 56, 57). Such thalli may develop zoosporangia or resting spores, and

it is not uncommon to find them intermingled at the edge of the substratum.

The formation of endoöpercula is fundamentally the same as in *K. granulata* (FIG. 53) and need not be described further. Both exo- and endoöpercula may occur on the same sporangium (FIG. 52). During dehiscence the operculum is pushed up by the zoospores and may remain attached at one side of the exit orifice or is carried away. Normally, the first discharged zoospores form a globular mass at the orifice and then disperse in a short time while the remainder of them ooze out slowly. Fairly often, however, the initial globular mass is surrounded by a layer of dense matrix and may become quite large in diameter as more zoospores are discharged into it. In such cases the mass may suddenly rupture in localized regions, and the zoospores then stream out in long strands or tendrils (FIG. 58). The spores appear to be stuck together, and from 2 to 4 minutes may elapse before they separate and swim away. In other atypical cases the initial globular mass may not form, and the zoospores ooze out in long sticky strands.

Karlingia marylandia sp. nov. (FIGS. 70–78)

Fungus saprophyticus. Sporangiis laevibus, sphaericis, 20–60 μ, ovalibus, 20–72 × 30–85 μ, pyriformibus, 20–28 × 40–60 μ, oblongatis, elongatis vel irregularibus; 1–2 papillis exeuntibus vel tubulis, 10–26 × 15–204 μ. Rhizoidibus plerumque a pluribus locis in sporangiorum superficie emergentibus, principibus axibus plerumque crassis diametro attingentibus 12 μ, maxime ramosis et extensis. Operculo apiculato vel submerso in papillis exeuntibus vel tubulis, vadoso, figura ad modum patellae vel crateris usque ad 17 μ diametro. Zoosporis hyalinis, sphaericis, 5.5–6 μ, cum uno globulo refractivo hyalino, 2.3–2.8 diametro. Sporis perdurantibus ignotis.

Saprophytic in cellulosic substrata in soil and water from a ditch on the G. A. Stonesifer farm near Frederick, Md.

As is indicated in the above diagnosis, this species is similar to *K. granulata* and *K. lobata* in having endo- and exoöperculate sporangia and irregularly constricted rhizoids (FIG. 70), but it differs from them by the presence of a large hyaline refractive globule instead of numerous minute granules in the zoospores (FIGS. 76–78). In this respect its zoospores are similar to those of *K. hyalina,* but differ by their larger size. *Karlingia marylandia* differs

further from this species by having only 1 or 2 exit papillae and by the lack of conspicuous plugs of hyaline material in them. Furthermore, instead of papillae it may occasionally form exit tubes which may be 10 to 15 μ broad by 26 to 204 μ long (FIGS. 74, 75). Also, the sporangium wall is usually quite thick, 1.8–2.6 μ, in *K. marylandia* and turns brown in old cultures.

The development of endoöpercula in this species is fundamentally the same as in *K. granulata* and *K. lobata,* and they are never deep-seated in the exit papillae or tubes (FIGS. 70, 75). Nor are they developed as commonly as the exoöpercula. The latter are usually deeply bowl-shaped (FIGS. 71, 73), thick-walled, and may persist or be carried away by the discharging zoospores. In a few exceptional sporangia both exo- and endoöpercula were found at and in the same exit papilla (FIG. 72). In such cases an exoöperculum had apparently been formed and pushed up slightly without the subsequent discharge of protoplasm or zoospores, after which an endoöperculum developed lower down in the exit papilla, as reported previously for *K. granulata.*

Dehiscence of the sporangium and discharge of the zoospores usually takes place very slowly so that several minutes may elapse before the sporangium is emptied. As in the previously described species the emerging zoospores form a globular mass at the exit orifice which is at first surrounded by a thick layer of slimy matrix (FIG. 71). The latter expands and becomes progressively thinner as more zoospores emerge and finally disperses in the surrounding water without forming a vesicle. Most of the zoospores are from 5.5 to 6 μ in diameter (FIG. 76), but numerous smaller ones may develop in the same sporangium with the larger spores. These vary from 2 to 3.5 μ in diameter (FIGS. 77, 78) and have a correspondingly short, 18–20 μ, flagellum. Such zoospores degenerate in a short time without becoming actively motile.

Besides the six new species described above, *K. rosea,* the type species of the genus, was isolated from more than thirty soil collections from various parts of Maryland.

With the addition of these new members, *Karlingia* now includes ten species, four of which, *K. rosea, K. spinosa, K. hyalina,* and *K. chitinophila,* are strictly endoöperculate. Of the remaining six species *K. asterocysta, K. curvispinosa* and *K. dubia* are strictly

exoöperculate, while *K. granulata, K. lobata* and *K. marylandia* are both exo- and endoöperculate.

According to present interpretations, *Karlingia* is very similar and almost identical to *Rhizophlyctis* in general appearance, structure and development. The only fundamental difference is its operculate sporangia, which is a valid generic distinction. In *Karlingia* most of the sporangia and resting spores develop directly from the encysted zoospore, but in a low percentage of thalli they may develop as an enlargement of the germ tube. In the majority of thalli several rhizoidal axes are present, and these usually arise at several points on the periphery of the sporangium or resting spore. However, thalli with only two or even one rhizoid attached at or near the base occur fairly often. In *Rhizophlyctis,* on the other hand, development of the sporangia and resting spores as an enlargement of the germ tube has not been reported, but when these species have been studied intensively this type of development will probably be found to occur. Furthermore, thalli with a single rhizoidal axis have not been reported in *Rhizophlyctis.*

Thalli of exoöperculate *Karlingia* species with a single basal rhizoid are very similar to those of non-apophysate members of *Chytriomyces* (Karling, 1945, 1947), another genus of exoöperculate, monocentric, eucarpic chytrids with extra-matrical sporangia and resting spores, and the same fundamental type of development. The basic differences between the two genera at present are the number and position of the rhizoidal axes and the presence or absence of an apophysis. *Rhizophlyctis* is closely related to an inoperculate genus, *Rhizidium,* and differs from it only by the presence of several instead of a single rhizoidal axis. Obviously, the distinctions between many of the chytrid genera at present are not very sharp, and are often based on slight vegetative structural differences such as those noted above. When these genera are better known, several of them will doubtless be merged. Until that time the exact taxonomic position and relation of *Karlingia* to other similar operculate genera will remain uncertain.

SUMMARY

Six new species of *Karlingia* were isolated from soil and water from various parts of Maryland in 1948. Four of these species,

K. chitinophila, K. asterocysta, K. curvispinosa, and *K. dubia,* were isolated and grown on chitinous substrata and are chitinophilic. The other two species, *K. lobata* and *K. marylandia,* however, are non-chitinophilic and have been grown only on cellulosic substrata such as onion skin, grass leaves and cellophane. *Karlingia chitinophila* is strictly endoöperculate, while *K. asterocysta, K. curvispinosa,* and *K. dubia* are exoöperculate. On the other hand, *K. lobata* and *K. marylandia* are both exo- and endoöperculate.

All of these species have the characteristic type of development and thallus structure of other species of *Karlingia,* and differ specifically by differences in size of zoospores and character of the resting spores.

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EXPLANATION OF FIGURES

FIGS. 1–8, *Karlingia chitinophila*. FIGS. 1, 2, spherical and oval zoospores with numerous minute, hyaline granules. FIGS. 4–7, variations in size and shape of resting spores, and the character of the wall. FIG. 8, germination of resting spore.

FIGS. 9–19, *K. asterocysta*. FIGS. 9–11, asteroid and verrucose resting spores. FIGS. 12, 15, polyrhizoidal sporangia with low inconspicuous exit papillae. FIGS. 13, 14, exit papillae of small sporangia. FIG. 16, initial stage of dehiscence. FIG. 17, zoospores swarming in a vesicle. FIG. 18, rupture of vesicle and escape of zoospores. FIG. 19, swimming zoospore.

FIGS. 20–35, *K. curvispinosa*. FIG. 20, spiny resting spore with a faintly visible, delicate boundary. FIG. 21, variations in size and shape of pegs and spines on resting spores. FIG. 22, large spherical resting spore with curved pegs or spines. FIGS. 22, 23, echinulate and smooth resting spores. FIG. 25, spherical resting spore with closely crowded or fused pegs. FIGS. 26, 27, 28, stages in the formation of the pegs or spines on the resting spores. FIGS. 29–33, variations in size and shape of sporangia. FIGS. 31–33, persistent post-cleavage stage. FIG. 34, dehiscence and discharge of zoospores. FIG. 35, swimming zoospores.

FIGS. 36–51, *K. dubia*. FIG. 36, polyrhizoidal thallus with constricted, thick-walled rhizoids. FIGS. 37, 38, exit papillae with separated and pushed up opercula. FIG. 39, exit papilla of empty sporangium. FIGS. 40, 41, initial and advanced stages of dehiscence. FIG. 42, zoospores with coiled flagella. FIGS. 43, 44, oval, spherical and elongate zoospores. FIGS. 45, 46, 47, thick-walled, constricted and coiled rhizoids. FIGS. 48–51, encrusted, verrucose and smooth resting spores.

FIGS. 52–69, *K. lobata*. FIG. 52, polyrhizoidal thallus with thick, wrinkled sporangium wall and exo- and endoöpercula. FIG. 53, endoöperculum under deliquesced tip of exit papilla. FIG. 54, portion of a thick-walled, constricted rhizoid. FIG. 55, monorhizoidal thallus with an exoöperculate, wrinkled sporangium. FIG. 56, stalked endoöperculate sporangium. FIG. 57, young stage in the development of a stalked sporangium. FIG. 58, zoospores escaping in sticky strands from globular mass at orifice of exit papilla. FIG. 59, swimming zoospore. FIGS. 60, 61, elongate and amoeboid, creeping zoospores. FIGS. 62–69, variations in size and shape of resting spores.

FIGS. 70–78, *K. marylandia*. FIG. 70, polyrhizoidal thallus with appendiculate, endoöperculate sporangium. FIGS. 73, 71, initial and advanced stages of dehiscence. FIG. 72, monorhizoidal thallus with sporangium which has formed two successive opercula. FIGS. 74, 75, sporangia with long irregular exit tubes. FIG. 76, normal sized zoospores. FIGS. 77, 78, minute zoospores.

THE TAXONOMIC SIGNIFICANCE OF SPOROGENOUS BASAL CELLS IN THE UREDINALES [1]

M. J. THIRUMALACHAR AND GEORGE B. CUMMINS

Sporogenous basal cells in the uredia and telia of the plant rusts have been emphasized in recent years as structures of considerable taxonomic significance. A review of the genera *Chaconia, Chrysocelis, Scopella,* and *Maravalia,* together with synonymous genera, indicates that the importance of basal cells has been overemphasized.

The development of teliospores in clusters on basal cells was recognized by Juel (3) when he established the genus *Chaconia.* The genus *Olivea* Arth. develops similarly. Mains (6), in establishing the genus *Scopella,* utilized the presence of well-developed, laterally free basal cells as a characteristic of primary significance. The genus *Scopellopsis,* established by Ramakrishnan and Ramakrishnan (9), was segregated from *Maravalia* because of the presence of basal cells and paraphyses. In this method of sporulation the spores develop successively from a basal cell. Consequently, the spores in any one cluster are of unlike ages, as pointed out by Juel (3). In the genus *Puccinia* Kuhnholtz-Lordat (4) has designated this method of development as the fasciculate type.

It is frequently difficult to demonstrate the method of sporulation with certainty. In the telia of *Maravalia achroa* (Syd.) Arth. & Cumm., *Scopella cryptostegiae* (Vestergr.) Cumm., *Puccinia solmsii* P. Henn., *P. bottomleyae* Doidge, and *Xenostele indica* Trium. the basal cells are not conspicuously elongate and consequently are easily overlooked. This is not the case with *Scopella echinulata* (Niessl) Mains, *Sorataea amiciae* Syd., *Hapalophrag-*

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mium milletiae Syd., *Desmella superficialis* (Speg.) Syd., and *Achrotelium lucumae* (Arth. & Johnst.) Cumm., species in which basal cells are elongate and readily demonstrable. In a third group of species, including *Scopella lucumae* (Diet.) Cumm., *Achrotelium ichnocarpi* Syd., and *Bitzea ingae* (Syd.) Mains, the teliospores are also clustered on basal cells but the latter are extremely difficult to observe because they are neither elongate nor laterally free. It is possible, using unrelated genera, not only to demonstrate the wide occurrence of basal cells, but also to prove the presence of intergradation.

The difficulties attending attempts to employ basal cells as structures of generic significance were discussed by Cummins (1) as follows: "Basal cells, as other morphological structures, are presumably subject to considerable variation. There is no rule by which one can definitely decide when basal cells cease to be basal cells and become parts of a cellular hymenium. This question does not arise in connection with the highly developed basal cells of *Scopella echinulata* (Niessl) Mains. It begins to enter when one studies *S. cryptostegiae* and *S. bauhiniicola,* however."

During this study telia of several species in different genera were examined for the presence of basal cells. These, together with species recorded or illustrated in the literature, but not previously mentioned in this paper, are listed below.

Name of rust	Authority	Development
Coniostelium quitensis	Sydow (10)	Prominent
Prospodium perodiosum	Cummins (2)	Prominent
Puccinia cynodontis	Kuhnholtz-Lordat (4)	Prominent
Puccinia polygoni	Kuhnholtz-Lordat (4)	Prominent
Sphenospora kevorkianii	Linder (5)	Prominent
Sphenospora yurimaguasensis	Present study	Prominent
Uromyces aleuropidis-repentis	Nattrass (8)	Prominent
Uromyces proeminens	Nattrass (8)	Prominent
Uromyces salsolae	Nattrass (8)	Prominent
Uropyxis amorphae	Present study	Prominent
Chrysopsora gynoxidis	Present study	Distinct
Maravalia achroa	Present study	Distinct
Mehtamyces stereospermi	Mundkur & Thirumalachar (7)	Distinct
Ravenelia spinosa	Present study	Distinct
Scopellopsis dalbergiae	Ramakrishnan & Ramakrishnan (9)	Distinct
Mainsia epiphylla	Present study	Obscure

In most of the rusts listed above the presence of basal cells has not been employed taxonomically but in the genera *Scopella, Chaconia, Scopellopsis,* and *Coniostelium* the basal cell provides the principal diagnostic character. Basal cells, together with more

important characters, are described in the genera *Tegillum, Desmotelium, Prospodium,* and *Olivea.*

The question arises as to how far the use of basal cells in the segregation of genera is justified. The structure obviously is variable and may be interpreted quite differently by different investigators studying similar material. If genera are to be differentiated because of the occurrence of basal cells a multiplicity of new genera for closely related rusts may result. Since the fasciculate type of development is now known to occur in unrelated genera it is doubtful if it should be accorded generic significance. We prefer to accept Kuhnholtz-Lordat's (4) conclusion that it merely represents one mode of spore development.

The telium is universally accorded primary importance in the characterization of genera of the Uredinales. It is our opinion that the spermogonium, an organ at least as conservative to change as is the telium, also provides a significant diagnostic character. There is little evidence that the spermogonia vary, within a genus, in their position relative to the epidermis. Exceptions apparently exist, however, in *Ravenelia* and *Melampsora* as currently constituted. A similar statement applies to telia, again with exceptions in *Ravenelia.*

Inasmuch as we consider that the presence of basal cells is not of generic significance the following key, based on other characters, is given to the group of genera in which basal cells have figured prominently.

Telia subepidermal or intraepidermal; teliospores one-celled, hyaline, germinating immediately by an external basidium; differentiated germ pore wanting.

Pycnia subcuticular
    Teliospores sessile ....*Chaconia* Juel.
                       (*Ypsilospora* Cumm.)
                       (*Bitzea* Mains.)
    Teliospores stipitate ....*Scopella* Mains.
Pycnia subepidermal
    Teliospores sessile ....*Chrysocelis* Lagerh. & Diet.
    Teliospores stipitate ....*Maravalia* Arth.
                       (*Argomycetella* Syd.)
                       (*Scopellopsis* Ramak. & Ramak.)

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THE GENUS HETEROCHAETE IN THE UNITED STATES

SISTER MARY CECILIA BODMAN

(WITH 4 FIGURES)

The tremellaceous genus *Heterochaete* was described by Patouillard (4) from material which Lagerheim had collected in Ecuador in 1892. The genus is essentially tropical or subtropical, and has been collected in or near the torrid zone in all parts of the world.

Fourteen collections have been recorded from the United States. The first of these was made by Langlois, in 1897, at Saint Martinville, Louisiana. Five other collections have been made in Louisiana, and six in Florida. One of the remaining collections is from Kentucky; the other from the District of Columbia. Burt regards the collection from the District of Columbia, which was made on the grounds of the United States Department of Agriculture, as an import, and the species he erected for it—*H. Shearii* (Burt) Burt—has been reported only once since, this time from Guam (1). The southwestern part of Kentucky is included in the old gulf embayment, in which the remnants of a tropical flora compete with oak-hickory forests, and with stands of beech and hard maple. A single specimen of *H. andina* was collected at Clinton, in this region.

Patouillard (2) established the species *H. sublivida* for the first collection made in the United States, that of Langlois in 1897. All other collections made in Louisiana have been determined as *H. andina*. One of the Florida collections, made near Miami by Thaxter, was determined by Burt as *H. andina,* and I have tentatively, on the basis of external appearance and the general aspect of the hymenium, assigned to the same species two collections from Florida which are a part of the Ellis Collection in the New York Botanical Garden. I have also determined as *H. andina* a collection made by C. L. Shear in Florida in 1937. Two collections made by R. Singer at or near Miami in 1942 are described in this paper as representing a new species.

Two types of fructification are found in the genus. One is distinctly gelatinous, appearing horny when dried. This type has not been reported from the United States, although the conspicuous *H. gelatinosa* (Berk. & Curt.) Pat. (4) has been found in Mexico. The second type, which characterizes all specimens thus far found in the United States, is coriaceous or farinaceous in aspect. All of these collections are moderately thick (more than 75 μ when dried), conspicuous, and heavily incrusted with mealy granules. The pegs are sometimes visible to the naked eye, and may always be seen if the specimen is examined with a hand lens. Some *Heterochaetes* have been identified as *Odontia*, *Hydnum* or *Hymenochaete*. It seems quite possible that if tropical collections of these genera are reexamined, some representatives of *Hetero-chaete* may be found.

I am indebted to Mr. John A. Stevenson, of the U. S. Department of Agriculture, for the loan of the type of *H. Shearii;* to Dr. H. S. Jackson, of the University of Toronto, for the specimen of *H. andina* collected by C. L. Shear, and to Dr. Donald P. Rogers, of the New York Botanical Garden, for the specimens from the Ellis collection. Dr. Rolf Singer, then of Harvard University, sent his Florida collections. It is with pleasure and with regret that I recall the courtesies extended to me at the Farlow Herbarium by Dr. David H. Linder. This work was done in the mycological laboratory of the State University of Iowa, under the direction of Prof. G. W. Martin.

Heterochaete Patouillard, Bull. Soc. Myc. Fr. **8**: 120. 1892.

Effused, resupinate, adnate, coriaceous to gelatinous, the hymenium pierced by irregularly distributed, infrequent or numerous sterile pegs consisting of fascicles of hyaline or faintly tinted parallel hyphae, compressed and partially gelatinized, arising from the subhymenium; subicular hyphae often extending beyond the hymenium to form a sterile, light-colored, villose margin. Aspect when dry, arid or somewhat farinaceous from the numerous granules which cover the surface, or horny; size and shape indeterminate, the fructifications often becoming confluent. Xeric, sometimes reviving to produce a second hymenium on top of the first. In section consisting of two distinct layers: a gelatinous, deeply-staining hymenium composed of basidia borne at or somewhat below the surface, of branching and contorted paraphyses, and

often of cystidia, or gloeocystidia, or both, and a subhymenium of interlaced, usually unbranched hyphae which may be gelatinized and indistinct or firm-walled, discrete, slightly refractive and tinted. A basal layer of parallel, more closely compressed hyphae sometimes present. Clamp connections occasionally observed. Basidia ovate or somewhat elongated, longitudinally or cruciately divided, bearing two or four epibasidia; spores suballantoid, with a prominent or sometimes small apiculus, germinating by repetition. Conidia rarely observed and then borne upon hymenial conidiophores.

Distinguished from all other resupinate, tremellaceous fungi by the presence of sterile hyphal pegs, which are usually conspicuous, numerous, and easily recognized.

The teeth or pegs that are found in other resupinate members of the Tremellaceae, for example in *Protodontia, Protohydnum,* and *Eichleriella,* are usually covered at least in part by the hymenium. Although spores may cling to the gelatinous parallel hyphae of the pegs of *Heterochaete,* those pegs are always completely sterile.

Proposed lectotype: *Heterochaete andina* Pat. & Lag. This species was the first described by Patouillard and Lagerheim. The material is adequate and is representative of the greater number of species that have been described. It appears also to be widely distributed.

KEY TO SPECIES

1. Fructification about 200μ in thickness, brown; hymenium brown in section ....2. *H. crassa*
1. Fructification less than 200μ in thickness, pale in color; hymenium colorless in section 2
   2. Fructification with a pinkish tinge, usually less than 100μ in thickness; spores $10–18 \mu$ in length ....1. *H. andina*
   2. Fructification not at all pink, usually $100–200 \mu$ in thickness 3
3. Spores $14–17 \mu$ in length; gloeocystidia present; pallid except for colored basal layer ....3. *H. Shearii*
3. Spores $8–12 \mu$ in length; gloeocystidia absent or inconspicuous; fructification dull, without colored basal layer ....4. *H. sublivida*

1. Heterochaete andina Pat. & Lag., Bull. Soc. Myc. Fr. **8**: 120. 1892.

Fructification thin, usually $50–100(–150) \mu$, surface dull, covered with coarse, mealy granules, pinkish buff to pinkish cinnamon, occasionally cartridge buff; pegs blunt, rather thick, usually

darker than the hymenium, hyphae appearing brownish under the microscope and slightly gelatinized; subhymenium indistinct, gelatinized, colorless, occasionally bearing clamp connections; hymenium often appearing to rise directly from the substrate, composed of characteristic gelatinized paraphyses, basidia and cystidia; gloeocystidia occasionally present, colorless, sometimes clustered and numerous; basidia two- or four-celled, ovate, $15-20 \times 6-8\,\mu$; spores allantoid or suballantoid, $(10-)14-17(-18) \times (4-)5-6 (-8)\,\mu$. Conidia (?) occasionally observed.
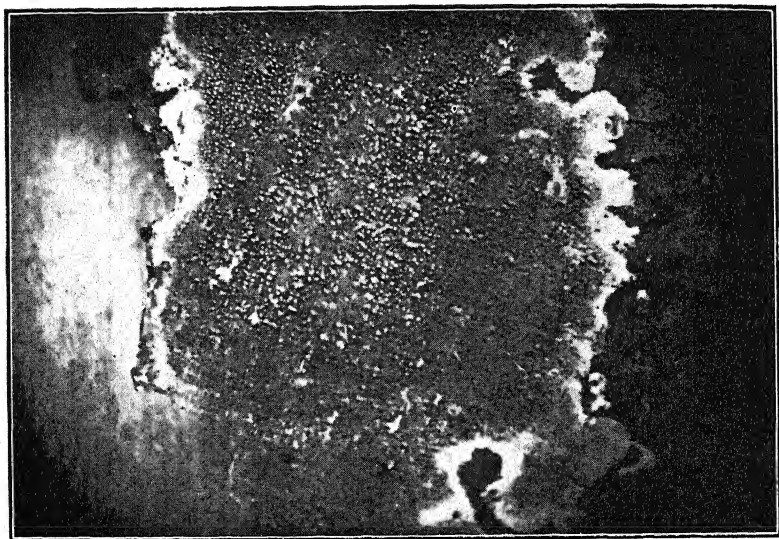
Fig. 1. *Heterochaete crassa*, $\times 4$. (Type)

Type locality: Ecuador.

Distribution: Kentucky, Florida, Louisiana; Puerto Rico, Costa Rica, Mexico, Panama; Ecuador, Argentina, Brazil.

Specimens examined:
Ecuador, Cotocallao, 1892. Lagerheim. FH. TYPE.
Florida, Cocoanut Grove. Thaxter, *93*. FH.
Florida, Highlands Hammock, 1937. Shear, *244*. Tor.
Florida. Calkins, *663* and *664*. NYBG.
Louisiana, St. Martinville, 1899. Langlois, *2855, 2988* and *ah*. FH.
Louisiana, New Orleans, 1908. Earle. NYBG; FH.
Kentucky, Clinton, 1925. *9262*. FH.

2. Heterochaete crassa sp. nov.

Fructificationibus resupinatis, primo orbicularibus, deinde latissime effusis, confluentibus, aridis in statu sicco, farinaceis, brunneis, dense setulosis; in margine albidis claris, villosis; setulis concoloratis, 160 μ altis; in sectione 150–400 μ crassis; hyphis subhymenialibus haud ramosis, distinctis, non gelatinosis, subcoloratis, minime zygodesmatibus, 2.5 μ diametris, accensis a subiculo tenue, hyalino; hymenio gelatinoso, in sectione fulvo; paraphysibus contortis, ramosis; gloeocystidiis typicis, hyalinis; cystidiis non visis; probasidiis ovatis, 19–21 × 11 μ, cruciatim partitis; maturis 18–27 × 10–15 μ; sporis 14 × 8 μ.
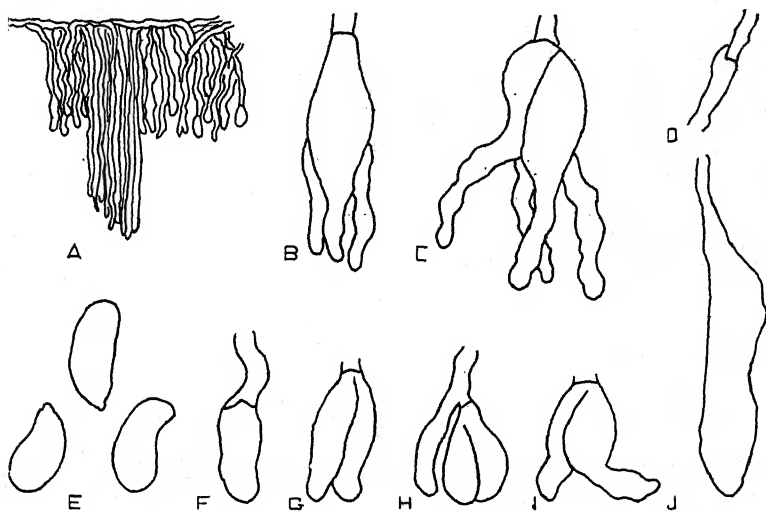
FIG. 2

a. Heterochaete andina, cross-section of fructification, ×170; *b, c, f–i.* Basidia (*b, c.* Type); *d.* Hypha with clamp connection; *e.* Spores; *j.* Gloeocystidium. All × 1000.

Fructifications 150–400 μ in thickness, Verona brown to cinnamon brown in the herbarium, walnut brown when fresh, pegs tapering slightly to the apex, concolorous with the hymenium, surface dry and farinaceous, the entire hymenial surface heavily incrusted with granules that cling to paraphyses, basidia, spores, and the hyphae of the pegs; margin white or light-colored, mycelioid, contrasting conspicuously with the dark hymenium; hyphae of the subhymenium discrete under the microscope, not at all gelatinized, 2.5 μ in diameter, unbranched, very faintly tinted; clamp connections occasionally seen; basal layer of finer thin-

walled hyphae, colorless, compressed; hymenium and pegs after treatment with KOH a clear, golden brown; paraphyses contorted, branching; gloeocystidia colorless; no cystidia seen; probasidia oval, $19–21 \times 11\,\mu$; basidia four-celled, when mature $18–27 \times 10–15\,\mu$.

Type locality: Florida.

Distribution: Known only from the type locality.

Specimens examined:
  Florida, Miami, 1942. Singer, *1494*. FH. TYPE.
  Florida, Matheson Hammock, 1942. Singer, *1355*. FH.

The robust form, dark color, and hymenium appearing deeply colored under the microscope make this fungus seem conspicuously different from any other species found in the United States.

3. HETEROCHAETE SHEARII (Burt) Burt, Ann. Missouri Bot. Gard. **8**: 377. 1921.

Sebacina Shearii Burt, Ann. Missouri Bot. Gard. **2**: 758. 1915.

Surface dull in color, pruinose or farinaceous; texture coriaceous, dull white, drying olive buff; pegs scarce in the type specimen, darker than the hymenium, made up of a central sheaf of brownish hyphae which arise in the layer next to the substratum; subhymenium of distinct, lightly tinted hyphae in which no clamp connections have been observed; hymenium composed of branching paraphyses, basidia located somewhat below the surface, and conspicuous and rather numerous gloeocystidia, which exceed the basidia but which never rise above the surface; basidia approximately $15 \times 9–10\,\mu$; spores $9–15 \times 4.5–6\,\mu$.

Type locality: District of Columbia.

Distribution: District of Columbia; Guam.

Specimens examined:
  District of Columbia, Washington, 1902. Shear, *1238*. USDA. TYPE.
  Guam, 1919. Edwards, 10778. Herb. MBG; FH.

There are so few of the characteristic hyphal pegs present in the type specimen that four or five sections may be mounted without a single peg being seen. It is not surprising that Burt at first described this as a *Sebacina*. The pegs are more numerous in the specimen from Guam.

4. HETEROCHAETE SUBLIVIDA Pat., Bull. Soc. Myc. Fr. **24**: 2. 1908.

H. Burtii Bres., Ann. Myc. **18**: 51. 1920.

Fructification coriaceous, lightly incrusted with mealy granules, drab, the pegs inconspicuous and lighter in color than the hymenium, heavily incrusted; subhymenium inconspicuous, colorless; hymenium of typical paraphyses and basidia, no cystidia or gloeocystidia observed; basidia broadly ovate, (16–)20(–24) × 8–10 μ; spores 8–11 × 5–6 μ.

Type locality: Louisiana.

Distribution: Known only from the type locality.
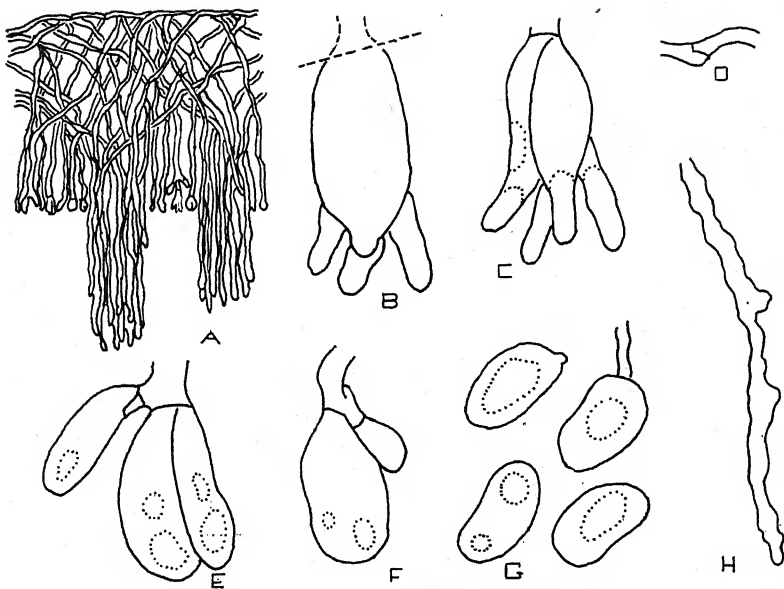
FIG. 3. (All drawings from type collection)

a. Heterochaete crassa, cross-section of fructification, × 60; *b, c, e, f.* Basidia; *d.* Detail of hypha, showing clamp connection; *g.* Spores; *h.* Paraphysis. All × 1000.

Specimens examined:
Louisiana, St. Martinville, 1897. Langlois, *2882.* FH. *Cotype.*

This species is noticeably duller in color than is any other species occurring in the United States.

It will be noted that *H. andina, H. Shearii* and *H. sublivida* seem to intergrade, and there at once arises the question of whether or not these are good species. Not all three may be valid. Four or five characters appear to differentiate them: (1) The presence of gloeocystidia in *H. andina* and *H. Shearii,* but not in *H. sublivida;* (2) a pronounced pinkish tinge in *H. andina,* whereas the other two are more subdued in color; (3) an inconspicuous,
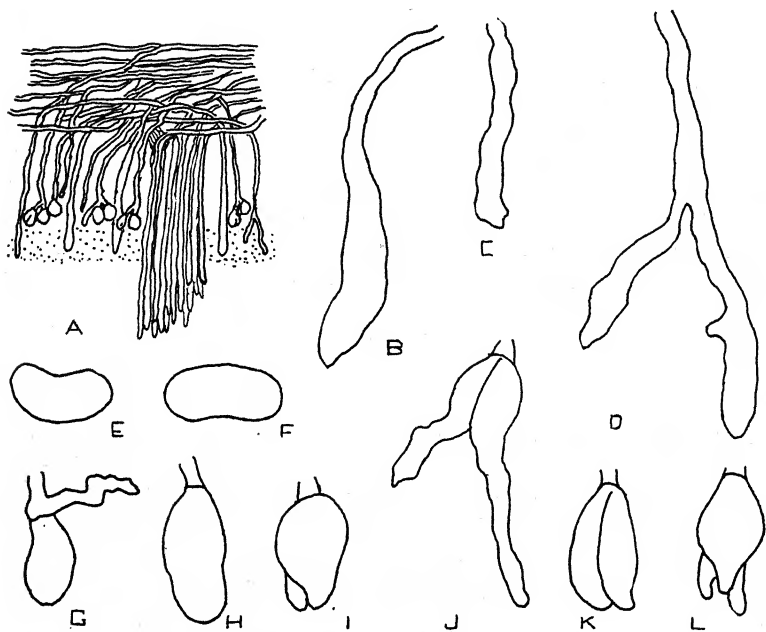
FIG. 4. (All drawings from type collection)

a. Heterochaete Shearii, cross-section, × 170; *b.* Gloeocystidium; *c. d.* Paraphyses; *e, f.* Spores; *g–l.* Basidia. All × 1000.

gelatinous, colorless subhymenium in *H. sublivida* and *H. andina,* contrasted with a firm, slightly-tinted, easily identifiable one in *H. Shearii;* and (4) a range of spore size in *H. andina* so wide that it includes also *H. Shearii* and *H. sublivida.* The basidia of *H. Shearii* are also smaller than are those of the other two. A complicating factor is that *H. sublivida* and *H. Shearii* have been

but rarely collected, and therefore accurate knowledge of the amount of variation in these two species is not available.

Color is certainly not a dependable species character, particularly when the color variation is merely a small difference in shade. Cystidium and gloeocystidium are terms in need of more precise delimitation. *H. Shearii* seems to possess both cystidia and gloeocystidia, although Burt in his vivid descriptions mentions only gloeocystidia. Neither cystidia nor gloeocystidia are mentioned for *H. andina* and *H. sublivida* and I have been able to find neither in *H. sublivida,* but long, slender, colorless organs arising beneath the hymenium and extending through it, occasionally becoming apparent above the surface, are easily demonstrated in almost every specimen of *H. andina.* In the specimen collected by F. S. Earle in New Orleans they seem to be clustered, and are very numerous. These I interpret as gloeocystidia. Patouillard in discussing the genus (3) remarks, "Parfois des cystides éparses ou groupées au sommet des émergences," but I have found nothing that will meet this description.. Attempts to segregate the species of this genus upon the basis of organs so poorly defined are, to say the least, precarious.

Other histological characters may be of more significance. The spores of *H. sublivida* seem to be noticeably smaller and plumper, the basidia somewhat larger than those of the other two species. In contrast with this, the structure of the subhymenium of *H. Shearii* seems much different from that of *H. andina* and *H. sublivida.* The smaller spores of *H. andina* have been found in only one collection—that of F. S. Earle in New Orleans. These are accompanied by small basidia, and by the numerous and conspicuous gloeocystidia that were previously mentioned. It does not seem unreasonable to suggest that when African and Asian collections are interpolated into the American series a continuous, smoothly-blending succession of characters which indicate a single species may be the result. However, further study of the type material, and of other collections from both hemispheres, is indicated before any decision is made.

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4. —— **et G. de Lagerheim.** Champignons de l'équateur. Bull. Soc. Myc. Fr. **8:** 120. 1892.

CATACAUMA SABAL CHARDON IDENTI-FIED IN THE UNITED STATES AND MEXICO

Donald P. Limber and Anna E. Jenkins [1]

(with 2 figures)

An ascomycete on three specimens of *Sabal* leaves taken in con-nection with plant-disease surveys of the United States Depart-ment of Agriculture (*cf.* 2 and 4) was identified as *Myriangium sabaleos* Weedon (5). Our further study of this myriangiale ap-pears in a companion paper (3). We are here presenting our more or less parallel study of a dothideale, *Catacauma sabal* Chardon, which we found in abundance on two of the specimens referred to above. Previously this has not been distinguished from the myriangiale, and to our knowledge *C. sabal* has not been reported from other than the type locality, which is the Dominican Republic.

Two specimens on which we identified the myriangiale were from Georgia (2), *viz.* on *Sabal palmetto,* Richmond Hill, De-cember 9, 1943, A. W. Blizzard *890;* and on *Sabal minor,* Savan-nah, December 31, 1943, A. W. Blizzard and Ilo Hein (Blizzard *943*). On the young green leaves from Richmond Hill we noted only the myriangiale (FIG. 1, *E* and *F*); on the other specimen, however, we soon discovered that another ascomycete, a dothideale, was present in abundance. The third specimen of diseased *Sabal*

taken during the survey was collected on *S. etonia* at De Soto City, Florida, by A. S. Rhoads, October 27, 1943 (4). On this specimen are the colorful ascomata of the myriangiale (FIG. 1, *G*), identified by Erdman West as *Myriangium sabaleos,* but much more numerous are the fructifications of the dothideale (FIG. 1, *C*). The same condition is true of the type specimen of *M. sabaleos*. This specimen also is from Florida, where it was collected on *S. palmetto* at St. Petersburg, February 13, 1923, by the author of the species.

That this fungus is widespread is further shown by three additional specimens now available. All are free from the myriangiale. One is an interception of diseased palm leaves from Mexico made at Brownsville, Texas, on April 27, 1946, by W. C. Edgeworth (Brownsville *61009*). This specimen was first examined by Flora G. Pollack, who had been present in 1944 when Blizzard *943* was examined by Limber and had been shown the preparations of both the dothideale and the myriangiale. Ascertaining that the fungus on the Mexican specimen was dothideaceous and that the conidial stage associated with it was the same as that on Blizzard *943,* Mrs. Pollack identified it as near *Phyllachora* or *Catacauma*. Since it seems to be the dothideale in question, we are suggesting that the palm may be a *Sabal*.

The other two additional specimens are both on *Sabal texana* from Brownsville, Texas, *viz.* November 17, 1945, Robert Runyon *4093,* and March 7, 1947, D. J. Smith. On the label of Runyon *4093* is essentially this statement: "It advances from the apex of the leaf toward the base, causing the leaf margins to die. It is only occasional on *Sabal texana*. In Brownsville, this palm is cultivated along sidewalks." Following Mrs. Pollack's examination (at Beltsville) of Brownsville *61009,* she also recognized the dothideale on Runyon *4093*. Mr. Smith's gathering, which we received while it was still fresh, was made at her behest. It was upon receiving this specimen of the dothideale that we actually undertook to identify it.

Pustules of the dothideale are abundant, especially so on the specimens of *Sabal texana* (FIG. 1, *A* and *B*). They are more conspicuous on the upper leaf surface and often do not penetrate to the lower surface, or are visible only as small blisters somewhat
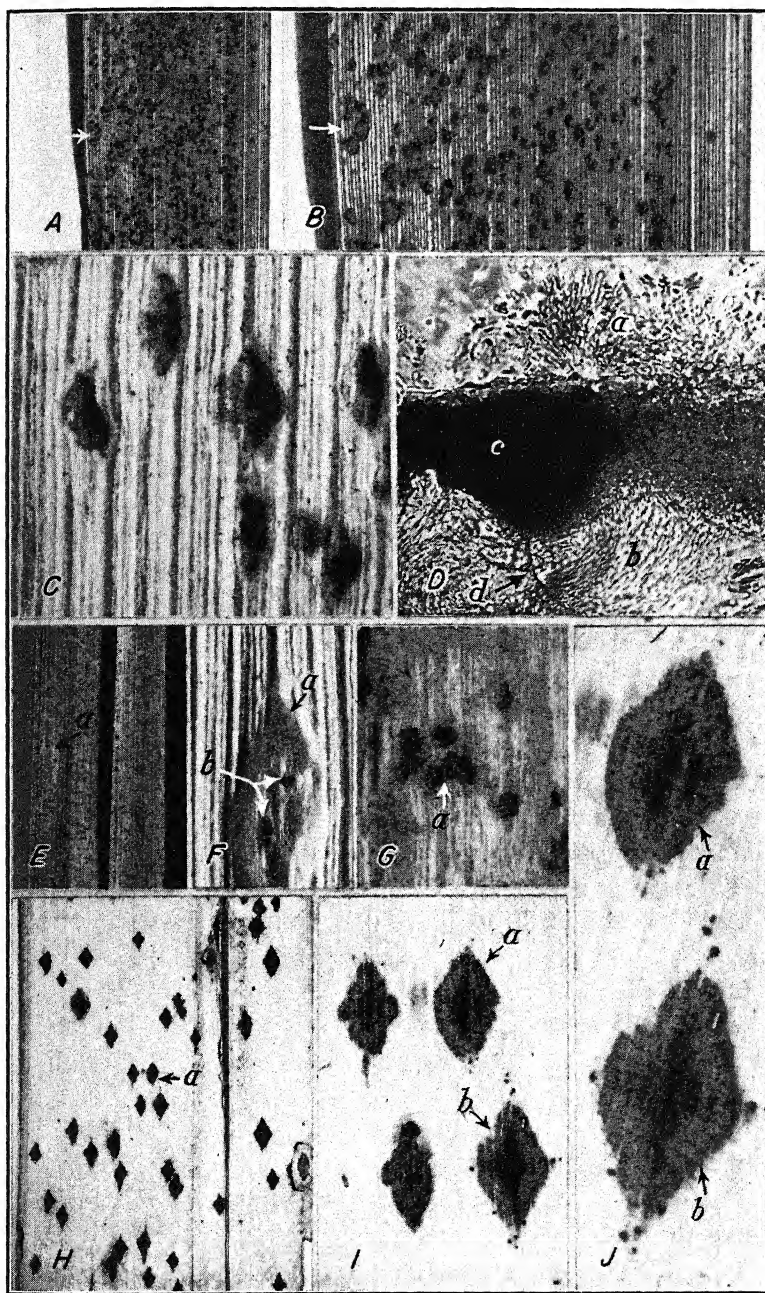
FIG. 1. *Catacauma sabal.*

paler than the surrounding leaf surface. Viewed en masse on the Texas specimens the pustules have a "seal brown" [2] cast. Individually they are pale to buffish brown, or black. The leaf tissue immediately surrounding the spot may not be discolored, or it may be whitish to brown. On the Rhoads specimen the spot often penetrates the leaf, and a pustule is present on each leaf surface.

Morphologically the dothideale is so different from the myriangiale [compare FIG. 2 with FIG. 2 (3)] that examined microscopically the two unrelated ascomycetes may be readily distinguished. We feel certain that Weedon's not having noticed the more abundant dothideale on the type specimen of her myriangiale, and likewise West's not having discovered it on the Rhoads specimen, is attributable to the similar superficial aspect of the two ascomycetes [compare FIG. 1, C, with FIG. 1, A (3)]. Where visible [FIG. 1, G, and FIG. 1, a (3)], stromata of the myriangiale are readily recognized by their red to purple color, such as "pomegranate purple" or "Bordeaux." Pustules of this fungus, where present, are interspersed haphazardly among those of the more abundant dothideale or the two may be in juxtaposition. In the young or poorly developed fructifications of either species, it may be difficult or practically impossible, even in microscopic mounts, to determine which is present or whether both are involved.

Our identification of the dothideale in 1947 was simplified by the fact that only the year before Chardon (1) had described as *Catacauma sabal* a fungus that he had collected on dead leaves of *Sabal umbraculifera* (Jacq.) Martius (det. L. H. Bailey, fide Chardon, letter July 17, 1948), in the Dominican Republic, July 11, 1937. To verify our determination we corresponded with Dr. Chardon, who wrote that the structure of the fungus and the measurements of the spore were in agreement with his description, and stated that the type was at Cornell University. The type was photographed for us under the direction of Clark T. Rogerson (FIG. 1, H–J) and subsequently examined at Ithaca by Limber.

The specimen consists of four leaf fragments—two faded nearly white, the other two tan. On the whitish fragments are the large

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[2] Colors quoted are based on Color Standards and Color Nomenclature, by R. Ridgway. 43 pp. Washington, D. C.

diamond-shaped spots shown in our figure 1, *H–J,* and as illustrated by Chardon (1, FIG. 14). On the two other leaf fragments are a few spots; however, the pustules are mostly of varying smaller sizes and crowded. In the sections made, some locules were filled with ascospores. This material was too old to distinguish more than the ascus walls. It was impractical, therefore, to look for the funnel-shaped depression above the pore of the ascus that we had noted on the fresh specimen from Texas (FIG. 2, *C*).

In some sections of the *Catacauma,* hyaline wefts of hyphae, possibly paraphyses, pass from the locules to the surface of the pustule (FIG. 1, *D*). Black, punctate incrustations, sometimes present on pustules of the *Catacauma,* consist of more or less radiating hyphae connected by a hyphal column to the stroma beneath. These have been examined many times and have always been found to be sterile.

In order to accommodate Chardon's description to the new details of morphology observed during our study of additional material, we append the following emended description.

CATACAUMA SABAL Chardon, Farlowia 2: 461, *figs.* 14–17. 1946, *emend.* Limber and Jenkins (FIGS. 1, *A–D* and *H–I,* and 2)

Stromata conspicuous, amphigenous (on *Sabal texana* concentrated on one leaf surface, usually the upper), slightly to abruptly raised, pale to dirty brown or black, en masse sometimes purplish in cast, visible below by a pale or yellowish fleck, 1–3 × 0.5–1.5 mm., main axis following that of the leaf, immediately surrounding tissue sometimes "pinkish buff" or browned without a definite marginal zone, mature stromata subepidermal or rarely in the mesophyll, consisting of a heavy black clypeus, with no stromatic tissue below or at the margin, and up to 10 labyrinthiform locules, 200–420 × 93–132 μ, which converge from the periphery toward the central locule; asci clavate, 8-spored, pedicel short, apex flattened and indented by a funnel-shaped depression leading to the pore (seen only in fresh material), 68–85 × 35–43 μ, with spores at first distichous, then crowded into the upper part of the ascus; ascospores 1-celled, hyaline, navicular, 25–36 × 7–12 μ, mostly 27–30 × 8–10 μ, with the contents slightly granular; paraphyses filiform, scarce; conidial stage occasionally present, acervuli subepidermal, erumpent, conidiophores hyaline, subulate, in a palisade, 4–7 × 3 μ; conidia hyaline, obclavate, 4.5–6 × 2–3 μ.
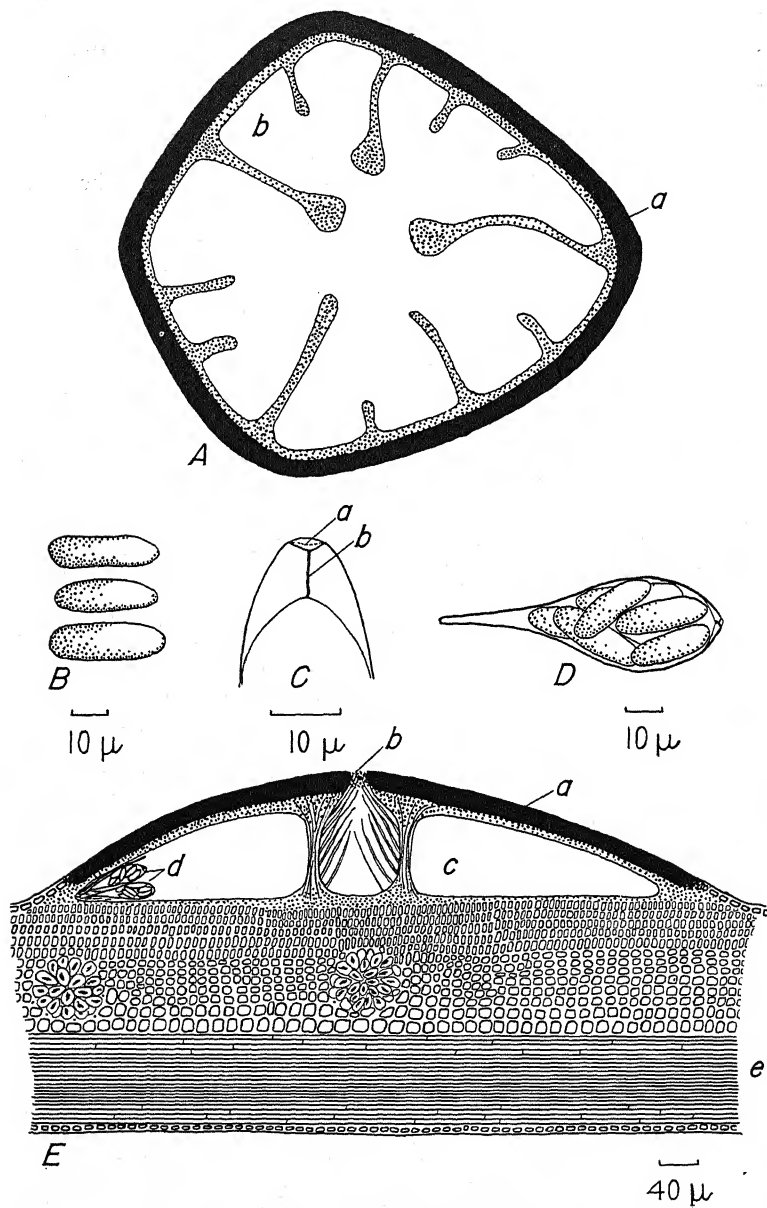
Fig. 2. *Catacauma sabal.*

Distribution and specimens examined:

Dominican Republic: On dead leaves of *Sabal umbraculifera* (Jacq.) Martius, Prov. Barahona, near the sulphur springs beyond Duvergé, July 11, 1937, Chardon (No. 760, Explorations of the Dominican Republic) (type). CU.[3]

Florida: On living leaves of *Sabal etonia* Swingle ex Nash, De Soto City, October 27, 1943, A. S. Rhoads. *Myriangium sabaleos* Weedon (5) also present on this specimen and previously identified as such (4). The *Catacauma* is much more abundant. USM.[4]

On the leaves of *Sabal palmetto* (Walt.) Lodd, St. Petersburg, February 15, 1923, A. G. Weedon. Although this is the type of *Myriangium sabaleos,* the *Catacauma* is the more abundant. NY, UI, USM.

Georgia: On green leaves of *Sabal minor* (Jacq.) Pers., Savannah, December 31, 1943, A. W. Blizzard *943. Myriangium sabaleos* also present and previously reported (2). USM, H.

Mexico: On palm (?*Sabal*) leaves, intercepted at Brownsville, Texas, April 27, 1946, by W. C. Edgeworth (Brownsville *61009*). USM.

Texas: On living leaves of *Sabal texana* (Cook) Becc., Brownsville, November 17, 1945, Robert Runyon *4093;* March 7, 1947, D. J. Smith. USM, H.

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    Bureau of Plant Industry, Soils, and Agricultural
        Engineering,
    Beltsville, Md.

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2. **Fields, W. S.** Summary of the more important plant diseases taken in connection with the insect and plant disease survey in the general vicinity of ports of entry from July 1, 1944 to December 31, 1944. Plant Disease Rptr. **29**: 181–183. 1945. [Processed.]

[3] Herbaria in which the specimens examined are deposited are indicated as follows:

CU   Herbarium, Department of Plant Pathology, Cornell University, Ithaca, N. Y.

NY   New York Botanical Garden, New York 58, N. Y.

UI   Herbarium, Department of Botany, University of Illinois, Urbana, Ill.

USM   Mycological collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md.

H   Pathological Herbarium, Bureau of Entomology and Plant Quarantine, Hoboken, N. Y.

[4] Duplicates of this specimen are being deposited in CU, NY, and UI.

3. Limber, D. P., and A. E. Jenkins. Weedon's *Myriangium* on *Sabal*.
    Mycologia **41**: 545–552. 1949.
4. Rhoads, A. S. Summary of observations on plant diseases in Florida
    during the emergency plant disease prevention project survey
    July 25 to December 31, 1943. Plant Disease Rptr. Sup. **148**:
    262–276. 1944. [Processed.]
5. Weedon, A. G. Some Florida fungi. Mycologia **18**: 218–223. 1926.

EXPLANATION OF FIGURES

Fig. 1. *A–D, Catacauma sabal* on leaves of *Sabal: A,* the dothideale in its general appearance on *S. texana,* Brownsville, Texas, March 7, 1947, D. J. Smith; ×1. *B,* part of *A;* × ca. 2½. *C,* several fructifications on the specimen of *S. etonia* from De Soto City, Florida, October 27, 1943, A. S. Rhoads; × 10. *D,* section of a fructification showing hyaline *Tubercularia*-like mass of hyphae (*a*) protruding from the ascus cavity beneath (*b*); *c,* clypeus; *d,* free ascospores; × ca. 225. *E–G, Myriangium sabaleos* for comparison with the *Catacauma. E,* inconspicuous spots on young leaf of *S. minor,* Richmond Hill, Georgia, December 9, 1943, A. W. Blizzard; ×1. *F,* single leaf spot from same source (*a*), showing ascomata (*b*); × ca. 8. *G, ascomata* (*a*) on the Rhoads specimen cited above; × 10. *H–J,* fructifications of the *Catacauma* on *Sabal umbraculifera,* Dominican Republic, July 11, 1937, Chardon *760* (*type*): *H,* ×1; *I,* same as *H, a,* × 5; *J, a* and *b,* same as *I, a* and *b,* × 10. Photographs by R. L. Taylor (*A–C* and *F* and *G*), by Limber (*E*), by M. A. Jaeger (*D*), and by W. R. Fisher (*H–J*).

Fig. 2. *Catacauma sabal. A,* horizontal section through a fructification showing the clypeus (*a*), and arrangement of the locules (*b*), as well as their incomplete separation; × ca. 100. *B,* three ascospores; × 500. *C,* apex of an ascus from the fresh specimen from Texas showing funnel-like depression (*a*), above the pore (*b*); × 1000. *D,* young ascus with spores; × 500. *E,* vertical section showing the location of the fructification beneath the epidermis: *a,* clypeus; *b,* ostiole; *c,* locule; *d,* asci in locule; *e,* leaf tissue; × 100. Drawings from Texas specimen by Limber.

WEEDON'S MYRIANGIUM ON SABAL

Donald P. Limber and Anna E. Jenkins [1]

(with 2 figures)

Weedon's *Myriangium sabaleos* (10) was described on the basis of a specimen she collected on leaves of *Sabal palmetto* at St. Petersburg, Florida, on February 15, 1923. In 1943 the fungus was again collected on *S. etonia* at De Soto City, Florida, by A. S. Rhoads (7, *p. 275*) and in Georgia on *S. palmetto* at Richmond Hill and on *S. minor* at Savannah, by A. W. Blizzard (2). The specimen from Richmond Hill shows only the myriangiale. The other two specimens of 1943, as well as the type specimen, were probably collected for the more conspicuous and much more abundant dothideale fruiting on them. Of these two parasites, only *Catacauma sabal* Chardon, as we have identified the dothideale (4), is known to cause any appreciable harm.

The actual leaf spots produced by the so-called *Myriangium* are usually small and insignificant. On the dry, young leaf fragments of the specimen of *Sabal palmetto* from Georgia, they are elongate, 0.25×1.3–5 mm., with the longer diameter paralleling the leaf axis. They are slightly thickened, minutely wrinkled lengthwise, pale, sometimes "pinkish buff," [2] with a definite margin.

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[2] Colors in quotation marks are based on Ridgway, R., Color Standards and Color Nomenclature. 43 pp. Washington, D. C.

Ascomata, or stromata, of the myriangiale develop on both leaf surfaces, although they are more abundant as well as more prominent on the upper side. Often sparse and scattered, they may be plentiful in limited areas appearing singly or in groups; more rarely a few may be coalescent (FIG. 2, *A;* also see 4, FIG. 1, *G*). They are circular to subcircular, elliptical or verruciform, reaching 225–750 μ long by 200–500 μ wide, and 65–200 μ thick. Stromata on the young leaf growth of *Sabal palmetto* from Georgia are whitish, but on the other older specimens they are distinguishable by their bright coloration, "carmine" to purple—"amaranth pur-
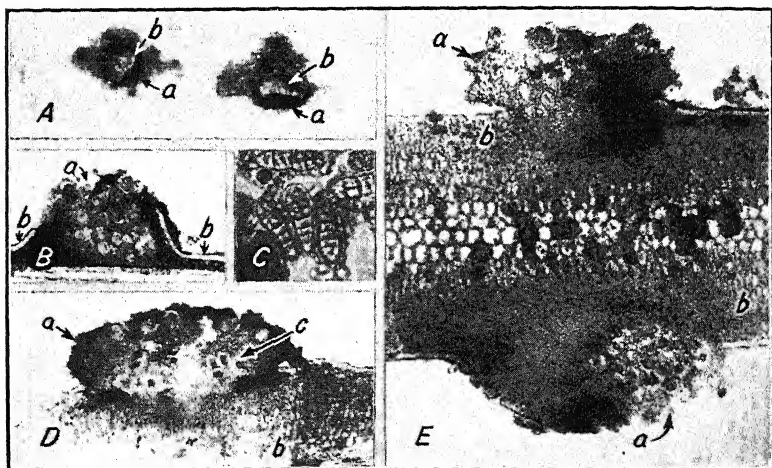
FIG. 1. *Myriangium* on *Sabal.*

ple," "pomegranate purple," or "burnt lake." Sections reveal that the ascomata form in or beneath the epidermis (FIG. 1, *B*) or in the mesophyll becoming superficial (FIG. 1, *A, b; B,* and *D*), although they may remain internal. Dense, hyaline hyphae within the leaf structure may lead from the base of one erumpent ascoma to that of another on the opposite side of the leaf (FIG. 1, *E*). Such a development in *Myrianginella tapirae* is reported by Stevens and Weedon (9, FIG. 7), who termed it a "double ascoma." The bright coloration of the ascoma is limited to the peripheral region. Within, the pseudoparenchymatous tissue is hyaline to dilute olivaceous. In some sections, however, the entire mass including

the ascospores becomes stained red to purple from the coloring matter in the outer part of the stroma.

The numerous asci are distributed irregularly throughout the stroma, but in external fructifications may be concentrated toward the outer part. The asci are subspherical to obovoid, 21–29 × 17–23 μ, and the gelatinous wall is noticeably thickened (FIG. 2, C). Ascospores are clavate, straight or curved, and muriform, with three to five transverse walls and one or two longitudinal septa (FIG. 2, E). In one instance an incomplete longitudinal septum was observed (FIG. 2, E) and such has been noted by Petrak (6) in *Myrianginella tapirae*. A layer of awl-shaped conidiophores bearing hyaline conidia (FIG. 2, D) has several times been seen in locules in the stroma (FIG. 2, F, b). From its close association with the myriangiales, it seems that it must belong to that fungus.

As co-author with Stevens in describing three similar species of myriangiales and discussing their position in the order on the basis of Theissen and Sydow's classification (8), Weedon was in a unique position to describe the similar myriangiale on *Sabal*. However, she was handicapped by the presence of the *Catacauma* in an old state of development and by the sparseness of the myriangiale. Her slide made from microtome sections shows that the ascoma of the myriangiale may become superficial. This character and the reddish to purple coloration of the ascomata would have placed the fungus in the genus *Myrianginella* created by Stevens and Weedon to describe their *M. tapirae*. They separated *Myrianginella* from *Myriangina* (Henn.) Hoehn. on the basis of the superficial development of the ascoma in *Myrianginella* and its intramatrical development in *Myriangina*.

In discussing the genus *Myrianginella* in 1927, Petrak (6) removed Stevens' basis for separating the two genera by reporting a superficial ascoma for *Myriangina mirabilis* (Henn.) Hoehn., type of *Myriangina*. This ascoma is illustrated by Jenkins (3, pl. 2, A), who noted also a copious development of asci in internal stroma of this species (3, p. 3). Calling attention to the fact that in *Myriangina mirabilis* the fructifications are pale yellow, Petrak disposed of *Myrianginella tapirae* by transferring it provisionally

to the genus *Uleomyces,* in which the fructification is also red to purple.

Rhoads (loc. cit.) alluded to Miller's (5) statement that *Myriangium sabaleos* "does not belong to" *Myriangium.*

Stevens and Weedon's account, together with Weedon's description of her myriangiale on *Sabal,*[3] led us to believe that Weedon actually intended to describe this in the genus *Myriangina.* When we referred to the type specimen, we found it labeled *"Myriangina sabaleos* n. sp. Weedon." The error represented in the published description was explained by Miss Weedon (Mrs. Amy G. Weedon Moore) upon our corresponding with her. She wrote:[4] "In the original manuscript as well as in the first typewritten copy the name appears as *Myriangina sabaleos.* However, on the copy sent the printer I find that the name has been changed to *Myriangium.* Who is or was responsible I do not know. The writing is not mine, nor does it look like Dr. Stevens'. The proof was evidently checked with this copy and thus the error slipped by."

Without further discussion of the genus *Myriangina* at this time, except to call attention to the large size of the ascus and ascospores as compared with those of *Myriangium sabaleos* (compare FIG. 2, *A* and *B,* with *E* and *C*), we are transferring this species to *Myrianginella.* In so doing we are not recognizing Petrak's provisional transfer of the type of *Myrianginella* to *Uleomyces,* but are revising the description of *Myrianginella.* We are also revising the description of *Myriangium sabaleos* in conformity with its morphological characters as we have observed them during the present study.

[3] This is here quoted for convenience of reference:

Myriangium sabaleos Weedon, sp. nov.

The stromata appear as small, black, slightly raised excrescences, 0.1–0.5 mm. in diameter; perithecia immersed, becoming erumpent, $300–900 \times 230–300\,\mu$; asci $95 \times 18–21\,\mu$, 8-spored; spores muriform, 3–5 transverse septa and 2–3 longitudinal walls, $21–26 \times 7\,\mu$.

On *Sabal palmetto,* St. Petersburg, Florida, Feb. 15, 1923, No. 2 (type). The black excrescences are surrounded by a pale zone, 1–2 mm. in diameter. The spots are visible from both sides of the leaf. The asci when first released are incased in a tough sheath, forming almost spherical bodies, $24–27 \times 25–28\,\mu$. These sheaths are frequently found in the Myriangiaceae.

[4] Letter of September 12, 1944, addressed to Jenkins.
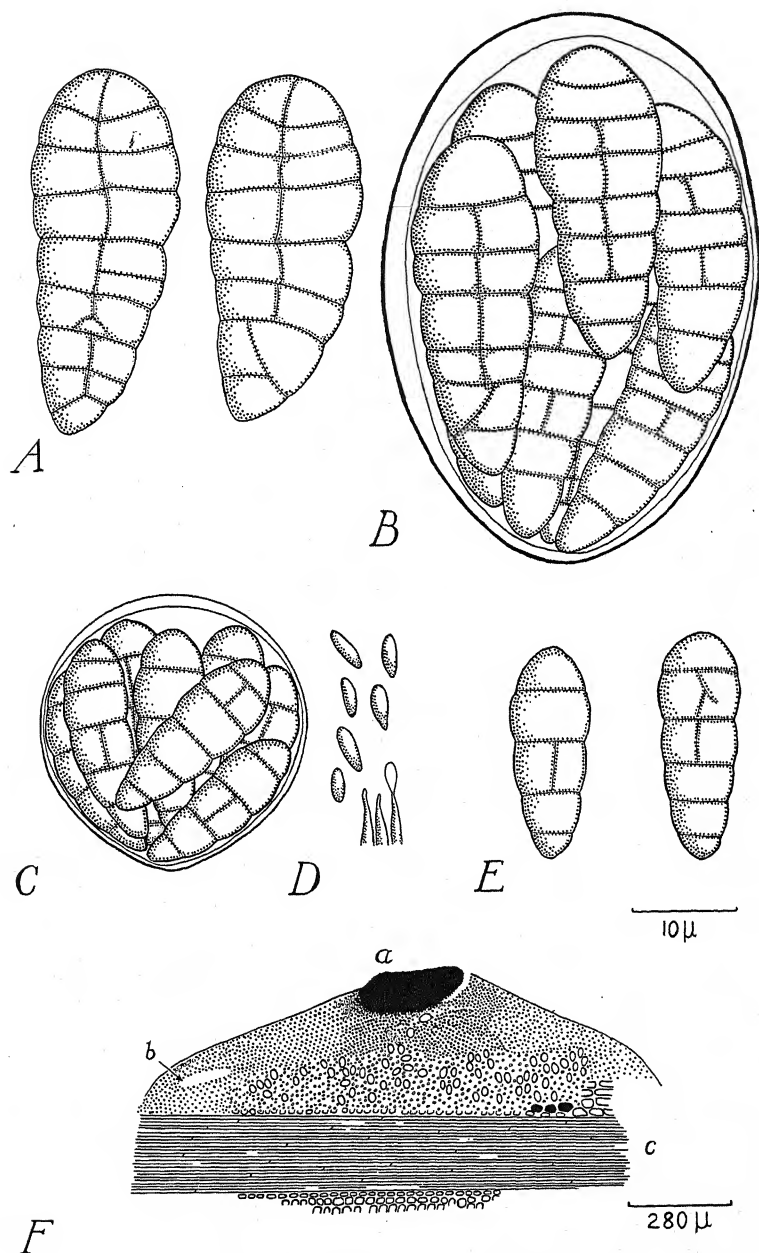
FIG. 2. *Myriangium* on *Sabal*.

MYRIANGINELLA Stevens and Weedon emend. Jenkins and Limber

Stroma developing within the host and erumpent, producing a superficial more or less irregular excrescence, with undifferentiated surface, exterior red to purple, external as well as internal stroma produced, light colored to olivaceous; asci at different levels, separated by pseudoparenchyma; spores muriform, hyaline.

Type: *Myrianginella tapirae* Stevens and Weedon (Mycologia **15**: 197. 1926).

Myrianginella sabaleos (Weedon) comb. nov., emend. Jenkins and Limber

Myriangium sabaleos Weedon, Mycologia **18**: 218: 1926.

Illustration: (FIGS. 1 and 2); Limber and Jenkins, Mycologia **41**, figure 1, *E–G*.

Spots amphigenous, small and inconspicuous to indistinct or negligible, usually somewhat paler than the surrounding leaf tissue, 0.25–1 × 1.3–5 mm., with the longest diameter parallel with the leaf axis, on dry specimen slightly swollen as well as often with fine longitudinal wrinkles, leaf tissue above developing ascomata becoming uplifted and variously ruptured, forming the clypeus; clypeus yellowish like the surrounding host tissue, or darkening to brown or nearly black, this coloration sometimes extending to the adjacent host tissue; ascomata amphigenous, more often epiphyllous, intraepidermal or produced in the mesophyll, at maturity innate to erumpent-superficial, in the latter case protruding from the clypeus or becoming completely exposed with the falling away of the clypeus; erumpent stromata circular, elliptical, or verruciform, in area 225–750 × 200–500 μ, and 65–200 μ thick; internal stroma extending horizontally 300–1200 μ, internal stroma and superficial stroma when present together reaching 132–430 μ in thickness, red to purple, in section coloration seems to be concentrated in the peripheral region of the pseudoparenchymatous structure, with the internal part hyaline to olivaceous, the so-called double ascoma sometimes formed by dense hyphae connecting the base of one exposed ascoma to another on the opposite side of the leaf; asci ovate, solitary in the tissue, distributed practically without order throughout the stroma although they may be concentrated in the outer part; asci, ovate, 8-spored, 21–29 ×

17–23 μ; ascospores clavate, straight or curved, muriform, with 3 to 6 transverse septa and 2 to 4 longitudinal septa, 16–27 × 5–9 μ, mostly 18–22 × 6.5–7.5 μ, hyaline; conidial stage observed in small openings in the stromata both in those that have not yet produced asci and those in which asci are present; conidiophores crowded, tapering, often flexuous toward the apex, 4–5 × 1–1.5 μ, those observed also on the surface of the stroma longer, 10–13 × 0.8–1.7 μ, hyaline; conidia elliptical to narrowly ovoid or clavate, 1-celled, 3–5 × 1.5–2 μ.

Associated with the myriangiale in the type specimen and apparently involved in the original description in so far as superficial aspects are concerned, but strictly not in microscopic characters, is the dothideale, *Catacauma sabal* Chardon (1). Its distinct nature was probably overlooked because of its overmature condition.

Distribution and specimens examined:

Florida: On living leaves of *Sabal etonia* Swingle ex Nash, De Soto City, October 27, 1943, A. S. Rhoads. *Catacauma sabal* Chardon most abundant as well as noticeable on this same specimen. USM.[5]

On leaves of *Sabal palmetto* (Walt.) Lodd., St. Petersburg, February 15, 1923, A. G. Weedon (*type*). The myriangiale is comparatively rare on this specimen, and *Catacauma sabal* much more abundant. NY, UI, USM.

Georgia: On leaves of *Sabal minor* (Jacq.) Pers., Savannah, December 31, 1943, A. W. Blizzard *943*. Associated as well as the most abundant fungus, *Catacauma sabal*. H, USM.

On young leaves of *Sabal palmetto*, Richmond Hill, December 9, 1943, A. W. Blizzard *890*. H, USM.

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    BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE,

        HOBOKEN, N. J., AND
    BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL
            ENGINEERING,
        BELTSVILLE, MD.

[5] Explanation of symbols is as follows:

USM   Mycological collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md.

NY   New York Botanical Garden, New York 58, N. Y.

UI   Herbarium, Department of Botany, University of Illinois, Urbana, Ill.

H   Pathological Herbarium, Bureau of Entomology and Plant Quarantine, Hoboken, N. Y.

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EXPLANATION OF FIGURES

FIG. 1. *Myrianginella sabaleos* on leaves of *Sabal: A,* surface view, showing up-tilted shield (*b*), and ascomata beneath (*a*), × 12.   *B,* section of an erumpent ascoma (*a*), seated in the epidermis (*b*), × 370. *C,* group of ascospores. *D, a,* section of an ascoma seated in the mesophyll (*b*); *c,* asci, × 100. *E,* section showing a "double" ascoma (*a a*), erumpent on opposite sides of the leaf (*b b*), × 100. *A–C,* and *E,* on *S. etonia,* De Soto City, Florida, October 27, 1943, A. S. Rhoads. *D,* on *S. palmetto,* Richmond Hill, Georgia, December 9, 1943, A. W. Blizzard *890.* Photographs by R. L. Taylor (A) and by Limber.

FIG. 2. Ascospores, *A,* an ascus, *B,* of *Myriangina mirabilis. C–F, Myrianginella sabaleos: C,* ascus; *D,* conidiophores and conidia frequently found in cavities in the stroma (*cf. F, b*); *E.* ascospores; *F,* diagrammatic representation of an ascoma, *a,* remnant of clypeus of host tissue, *b,* conidiabearing cavity, *c,* host tissue beneath fructification. *A–E,* 4 mm. = 1 μ; *F,* 1 mm. = 7 μ. Drawings by Limber.

STUDIES ON THE MORPHOLOGY AND CYTOLOGY OF THIELAVIA BASICOLA ZOPF [1]

GEORGE BLANCHARD LUCAS [2]

(WITH 1 FIGURE)

Thielavia basicola was first described in 1876 by Zopf (11) who found the organism in roots of *Senecio elegans* and lupines (12). It has been observed often on tobacco roots in association with *Thielaviopsis basicola,* the causal organism of black root rot. The pathogenicity of *Thielavia basicola* upon tobacco has not been demonstrated. Johnson (5) in 1915 believed *Thielavia basicola* to be the perfect stage of *Thielaviopsis basicola.* He reported a host range of nearly 100 species for *Thielaviopsis basicola* and found perithecia of *Thielavia basicola* in abundance upon *Cucumis maxima, Robinia pseudoacacia, Cytisus scoparius, Nicotiana tabacum* and to a lesser extent on numerous other hosts. McCormick (7) in 1925 showed that the two fungi were not related. She reported the occurrence of *Thielavia basicola* on the roots of tobacco, pea, violet and *Antirrhinum.* McCormick also showed that an isolate from violet roots, while exhibiting a tendency to form perithecia when grown in pure culture, was stimulated to produce perithecia abundantly when grown either with *Thielaviopsis basicola* or certain other fungi or with extracts from cultures of these fungi. In 1930, Emmons (1) described a soil-inhabiting species of *Thielavia* which readily produced perithecia in culture and which he named *T. terricola.* This fungus had been isolated originally by Gilman and Abbott (4) from Iowa and Louisiana soils and had also been observed on decayed strawberry roots in North Carolina (1).

The present discussion is concerned mainly with the morphology and cytology of an isolate of *Thielavia* from tobacco roots. Another from Austrian winter pea [3] and one from soybean [4] were also studied, to determine if strains of the fungus exist. All cultures were grown on several different artificial media and compared with an isolate of *Thielavia terricola* kindly furnished by Dr. C. W. Emmons.

MATERIALS AND METHODS

Tobacco roots infected with *Thielaviopsis* were collected and examined with a dissecting microscope for perithecia of *Thielavia*. Roots containing perithecia were plated on Richards agar (9) minus sucrose. Sterile filter paper was used as a carbohydrate source instead of sucrose. The medium tested about pH 5 after autoclaving. The plates were incubated at approximately 37° C. The above method proved very successful in isolating *Thielavia* from tobacco roots.

Single ascospore isolations were made with a Chambers micromanipulator. The spores were germinated in Van Tieghem cells on potato agar and transferred to tubes of oatmeal agar.

The perithecia of *Thielavia* used for sectioning and staining were produced in pure culture by growing the tobacco isolates on oatmeal agar in Petri dishes. Blocks of agar from 5- to 10-day-old cultures containing the perithecia were fixed in weak Flemming's solution and imbedded in paraffin in the usual way. Sections were cut $6\,\mu$ thick, stained with a 0.25 per cent aqueous solution of crystal violet and destained with picric acid and clove oil, according to the procedure recommended by Sass (10). Perithecia were also crushed and the clusters of asci stained with aceto-carmine or aceto-orcein. While this method proved very satisfactory in studying the increase in size of the ascus, nuclear structures were not well differentiated.

MORPHOLOGICAL VARIATION IN CULTURE

Forty-eight single ascospore cultures of *Thielavia* from the Austrian winter pea isolate were grown on oatmeal agar in test

[3] Isolated by Mr. Arthur Kelman of North Carolina State College.
[4] Isolated by Dr. S. G. Lehman of North Carolina State College.

tubes. All these cultures except one produced an abundance of fertile perithecia in 7 to 14 days. The exceptional culture had fluffy white aerial mycelium but no perithecia. It did produce a few scattered bodies which resembled perithecia but these never produced ascospores. When this culture was grown in the same Petri dish with other single-ascospore cultures no detectable fusion or stimulation was evident.

Nineteen single-ascospore cultures were obtained from an isolate of *Thielavia* from tobacco roots. All cultures produced an abundance of perithecia containing ascospores. Many additional mass isolates were obtained from tobacco roots over an eighteen-month period. In all cases the isolates produced numerous perithecia when grown on oatmeal agar.

Isolates of *Thielavia* from Austrian winter pea, soybean, tobacco and the culture furnished by Dr. Emmons were grown on Richards agar minus sucrose plus filter paper and incubated at room temperature. Under these conditions at the end of 10 days all cultures produced numerous perithecia scattered over the surface of the filter paper. Very little aerial mycelium was evident. The perithecia contained mature ascospores. At the same time duplicate cultures of all four isolates were grown on Richards agar plus sucrose but with no filter paper. At the end of 10 days all cultures had abundant white aerial mycelium. However, only the isolate from soybean had produced a sufficient number of perithecia to make them visible to the unaided eye.

The average size of the perithecia produced by the different isolates on various substrates varied widely. For instance, the perithecia produced by the soybean isolate on Richards agar plus sucrose averaged 110.5 μ in diameter. Perithecia produced by the same isolate on Richards agar minus sucrose plus filter paper averaged 219.9 μ in diameter. Perithecia of *T. basicola* found in tobacco roots averaged 59.7 μ in diameter, while perithecia produced by the tobacco isolate on Richards agar minus sucrose plus filter paper at room temperature averaged 130.8 μ in diameter.

All four isolates of *Thielavia* were also grown on oatmeal agar at room temperature. At the end of two weeks fifty mature ascospores from each of the four isolates were measured. At the end of two months fifty mature perithecia were measured. These

measurements as well as those of several other investigators are shown in table I.

TABLE 1

PERITHECIAL AND ASCOSPORE MEASUREMENTS OF *Thielavia* ISOLATES AS GIVEN BY VARIOUS WORKERS

Worker	Source	Substrate	Diameter of peri-thecia in microns	Size of spores in microns	
				Length	Width
Peglion (8)	Tobacco	Tobacco roots	80–100	8–10	4–5
McCormick (7)	Violet, tobacco pea	Violet, tobacco pea roots	99, 72, 66	10–13	4.5–6.5
Emmons (1)	Soil	Corn meal agar	80–125	10–16	7–9
Lucas	Tobacco	Tobacco roots	59.7	9.8–13.1	4.3–5.4
Lucas	Tobacco	Oatmeal agar	152.7	11.4–16.3	6.5
Lucas	Soybean	Oatmeal agar	162.5	11.4–13.1	6.5–8.2
Lucas	Austrian winter pea	Oatmeal agar	167.6	11.4–16.3	6.5
Lucas	Emmons culture	Oatmeal agar	96.7	11.4–13.1	6.5

CYTOLOGY OF THE PERITHECIUM AND ASCUS

The development of the perithecium of *Thielavia terricola* has been described by Emmons (2). The perithecia of the isolates of *Thielavia* from tobacco studied herein grew essentially as he describes. A hyphal coil forms which is soon surrounded by numerous hyphae. The interior of the growing perithecium is filled with slender, delicate hyphae, some of which are ascogenous hyphae containing paired nuclei. In the young perithecium, asci are found in all stages of development from crozier formation to mature asci. Frequently several asci in various stages of development can be seen growing from one hypha (FIG. 1, *C*). The ascogenous hyphae measure less than $3\,\mu$ in diameter when the croziers form. By the time the ascus is almost full grown, nuclear fusion has occurred and the ascus is characterized by a large fusion nucleus (FIG. 1, *E*) with a conspicuous dark-staining nucleolus. This nucleolus is approximately twice the diameter of the nucleolus seen in the ascogenous hyphae. Forty-five fusion nucleoli were measured. They ranged from 1.0 to $1.7\,\mu$ in diameter. The fusion nuclei were quite large, the average diameter being 4 microns. More than 200 asci were observed just after fusion had occurred, or in prophase I, but in no instance were chromosomes seen which measured over $2.0\,\mu$ in length. At this

stage it was not possible to distinguish clearly the number of chromosomes.

The nuclear divisions in the ascus seemed to follow each other rapidly. Relatively few figures of the first (FIG. 1, *F*) and second
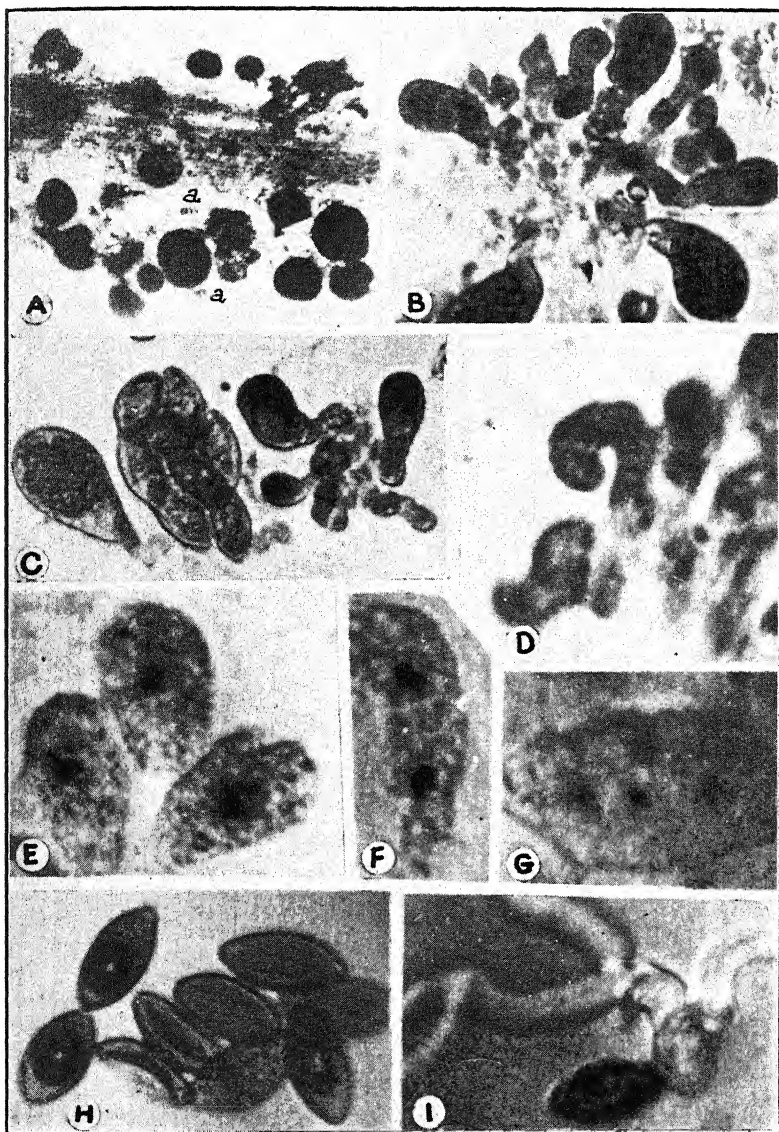
FIG. 1.   *Thielaviopsis basicola.*

(FIG. 1, *G*) division were seen as compared to the fusion nucleus stages. However, several figures were seen in which the chromosome number could be determined as more than three and less than six. One figure of the second division was seen where five dot-like chromosomes were visible. None of these measured over 0.5 μ in length. The divisions appeared to occur along any plane of the ascus. After the third division the nuclei seemed to be located near the periphery of the ascus. Cleavage furrows appeared in the cytoplasm of the ascus as the ascospores were delimited. After the ascospores are mature the asci appear to deliquesce, leaving the ascospores free in the perithecium.

<center>DISCUSSION</center>

It is evident that *Thielavia basicola* is widespread in the soil. Zopf reported it from Germany, Peglion (8) from Italy. It has been found in Connecticut, North Carolina, Louisiana and Iowa.

McCormick (7) found that isolates of *Thielavia* were stimulated to produce many perithecia in culture when grown with several other fungi or extracts from these fungi. The isolate studied by Emmons (1) produced perithecia in culture without the presence of other fungi or fungous extracts. On this basis and because of the difference in ascospore size Emmons assigned a new specific name to the fungus studied by him.

The observations herein reported indicate that *Thielavia basicola,* though homothallic, is composed of various strains or forms. The isolate from soybean, for instance, produced numerous perithecia on Richards agar plus sucrose. The other isolates did not. Perithecia produced by the Emmons isolate were on the average much smaller than the perithecia produced by the other three isolates. Perithecia of *Thielavia basicola* as found in tobacco roots were on the average smaller than those produced in pure culture. This was also true of the ascospores. Thus, both size and production of perithecia and size of ascospores appear to be dependent on the source of the isolate and the substrate on which it is grown. It is doubtful, therefore, if presence or absence of perithecia in pure culture or minor differences in ascospore size can be used as valid measurements for delimiting two species in the strains stud-

ied.   Very probably the isolate studied by Emmons should be included as a strain of *Thielavia basicola* and not given specific rank.

The association of *Thielavia basicola* and *Thielaviopsis basicola* is an interesting one.   The author has observed *Thielavia* only on those tobacco roots which had been infected with *Thielaviopsis*. This has been observed by previous investigators (3) and led to the assumption that *Thielavia basicola* was the perfect stage of *Thielaviopsis basicola*.   Attempts (6) to inoculate healthy tobacco roots with cultures of *Thielavia basicola* have not proved successful.   Yet, perithecia of *Thielavia* have been observed in the cortex of roots attacked by *Thielaviopsis*.   It may be that roots infected with *Thielaviopsis* are weakened or altered sufficiently to permit *Thielavia* to invade the tissues as a secondary organism.

SUMMARY

1. *Thielavia basicola* is widespread in the soil.   The species is composed of forms or strains.   The production and size of perithecia are dependent on the form isolated and the substrate on which it grows.   Ascospores found in tobacco roots are not as large on the average as those produced in culture.

2. Isolates of *Thielavia basicola* were obtained from tobacco roots.   Perithecia were produced in pure culture, sectioned and stained with crystal violet.   Very young to mature asci were seen. Crozier formation occurred.   The large fusion nucleus in each ascus contained a large nucleolus approximately twice the diameter of the nucleoli seen in the ascogenous hyphae.   Three divisions occurred in the ascus.   At prophase I the longest chromosome was not over $2\,\mu$ long.   At the third division the chromosomes were $0.5\,\mu$ or less in length.

3. Four isolates of *Thielavia* from different sources were grown and compared in culture.   Indications are that all four isolates should be included in the species *Thielavia basicola* Zopf.

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DESCRIPTION OF FIGURE

Fig. 1. *A*. Tobacco root affected with black root rot showing chlamydospores at *a* of *Thielaviopsis basicola* and perithecia of *Thielavia basicola* (approx. × 100). *B, C*. Developing asci (approx. × 900. *D*. Crozier formation (approx. × 1,800). *E*. Three asci each showing fusion nucleus with large nucleolus (approx. × 1,800). *F*. Ascus at first division two nuclei present (approx. × 1,800). *G*. Ascus at second division four nuclei present (approx. × 1,800). *H*. Mature ascospores (approx. × 900). *I*. Germinating ascospore (approx. × 900).

TWO UNUSUAL CONIDIAL FUNGI

Frederick A. Wolf

(WITH 5 FIGURES)

It occasionally happens, during the routine of collecting and identifying fungi, that a species having an unusual type of conidium and conidiophore is encountered. The two species given brief consideration herein are believed to be quite unlike any with which even well-trained and experienced students of fungi are acquainted, and consequently they are worthy of mention. One of them has been identified as *Cephaliophora tropica* Thaxter, and the other, as an hitherto undescribed species to which is given the name *Ypsilonia corticalis*.

CEPHALIOPHORA TROPICA

The genus *Cephaliophora* comprises two dung-inhabiting species, *C. tropica* and *C. irregularis,* the former being the type, both of which were described by Thaxter (1903). In the mycological literature available to the writer it is of more than passing interest that these species of *Cephaliophora* have never been recorded by other investigators.

C. tropica was isolated from materials sent from Jamaica, Java, Liberia, China, and the Philippine Islands. Presumably this species has a wide distribution in the tropics, and has been assumed to be restricted in range to the tropics. However, the isolates in hand appeared in cultures from inoculum obtained from decaying tobacco leaves which had long been in contact with the soil in a field at Oxford, N. C. On potato dextrose agar and other semi-solid media vegetative growth is profuse and fructifications are abundantly formed. The mycelium is loose, cottony, and septate. The conidiophores are erect, broadly-saccate, and have a short pedicel. Conidia are initiated as bud-like protrusions from the capitulum (FIG. 1). They extend outwardly in all directions and

are so abundant as eventually to conceal quite completely the capitulum, except its basal part and the pedicel (FIG. 3). Each conidiophore bears from 12 to 20 conidia, and all conidia are formed simultaneously. The conidia are broadly attached, ovoid
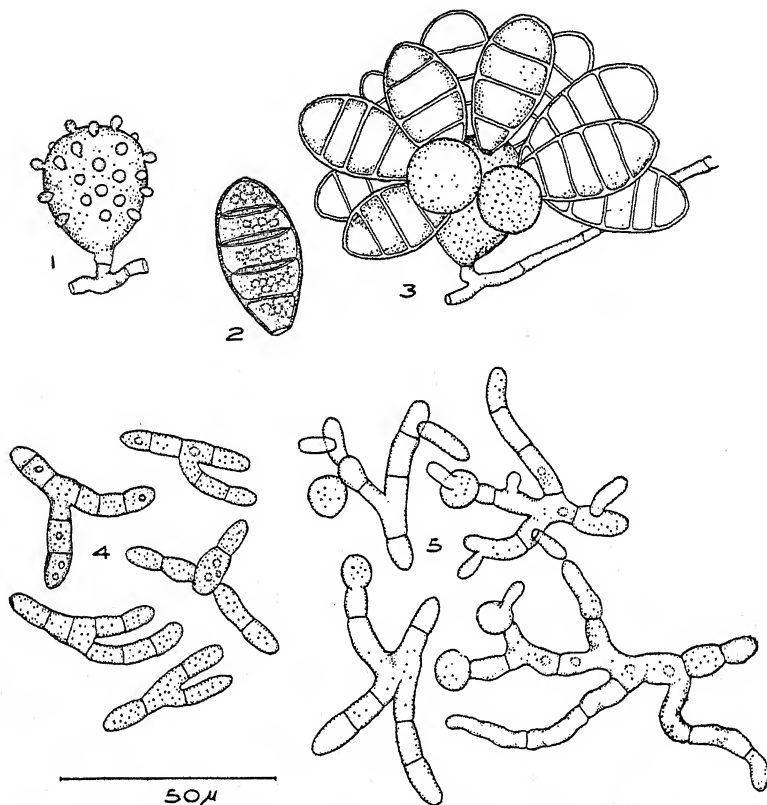
FIG. 1. Young conidiophore of *Cephaliophora tropica* bearing bud-like protrusions, the conidial initials.

FIG. 2. Mature conidium of *C. tropica*.

FIG. 3. Mature conidial fascicle of *C. tropica*.

FIG. 4. *Ypsilonia corticalis,* showing variations in shape of conidia.

FIG. 5. Germination of conidia of *Y. corticalis* in tap water, after 16 to 20 hours.

to elliptical, 3- to 5-septate, thick-walled, and yellow-brown (FIG. 2). Their average dimensions are $35 \times 16\,\mu$, but they may have an extreme length approximating $50\,\mu$.

The structural features of *C. tropica* show that it is among the Moniliales (Hyphomycetes), where it was assigned by Thaxter. It bears no marked resemblance, however, to *Oedocephalum* or other genera having capitate conidiophores. The conidia are not borne at the apices of small sterigmata, as occurs in *Oedocephalum*. Moreover the conidia of *C. tropica* are not thin-walled, as they are in other capitate hyphomycetes, but have an appearance not unlike that of conidia of *Helminthosporium*. In short, *Cephaliophora* is strikingly different and can be distinguished readily from all other capitate Moniliales.

Ypsilonia corticalis sp. nov.

Pycnidii 150–200 cr., sparsis, sphaericis, nigris, membraneis; contextu parenchymatico, conidiis ypsiliformibus, hyalinis, 3–5-septatis, 35–50 × 5–8 μ.

Hab. ad ramos emortuos *Quercus borealis,* socia *Lasiosphaeria pezizula,* in silvis, prope Durham, N. C., Amer. bor.

In *Ypsilonia* are included pycnidial fungi belonging to the Excipulaceae (Discellaceae) and possessing conidia resembling the shape of the Greek letter upsilon. Two species of *Ypsilonia* have been described previously, *Y. cuspidata* Lév. and *Y. vagans* Speg. Whether species of *Acanthothecium* and *Psalidosperma* should be regarded as members of *Ypsilonia,* as has been suggested, seems not to have been satisfactorily established.

The genus *Ypsilonia* was founded over one hundred years ago by Léveillé (**1846**), with *Y. cuspidata* as the type. This species was collected near Manila, P. I., where it occurred on the lower leaf surface of foliage of an unidentified member of the Anonaceae. *Y. vagans* was described by Spegazzini (**1908**). It occurred interspersed with *Zukalia vagans* Speg. (Perisporiales, Capnodiaceae) on the leaves of certain shrubs, including *Spiraea cantonensis* Lour., growing in the Botanical Garden at São Paulo, Brazil.

Ypsilonia corticalis occurs on the bark of oak slash in the Duke Forest. It is sparsely interspersed throughout patches of black turf, consisting of the conidiophores, and conidia, of *Helicoma curtisii* Beck., admixed with the perithecia of *Lasiosphaeria pezizula* (B. and C.) Sacc. Linder (**1929**) established the genetic

connection of this *Helicoma* and *Lasiosphaeria* by using cultures originating from ascospores.

The pycnidia are typically ypsiliform or bifurcate but the angle of divergence of the two branches may vary from acute to as wide as 180° (FIG. 4). They are hyaline, 3- to 7-septate, and measure 35–50 × 5–8 μ.

When placed in tap water or on maltose agar the conidia germinate readily (FIG. 5). The mycelial mat is adpressed, whitish, and lacks distinctive features. Conidia have not been observed to form in cultures.

Léveillé (1846) indicated that the conidia of *Y. cuspidata* lack septations but, because of the ratio of their length to width, it seems unlikely that mature conidia would remain non-septate.

The measurements of conidia of *Y. vagans* are 30–35 × 1 μ, and no mention is made of septation. In conidia so tenuous, septations would be difficultly discernible. In addition both conidia and conidiophores are ternate. Apparently the conidia arise from the apices of the ternate branches in *Y. vagans,* but from bifid branches in *Y. corticalis.*

Neither *Y. cuspidata* nor *Y. vagans* seems ever to have been collected more than once nor from any other than the type locality. The organism in hand is quite unlike either of these two species of Ypsilonia.

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STUDIES IN THE GENUS PLEOSPORA. I

Lewis E. Wehmeyer

(with 17 figures)

In a previous paper (10) an attempt was made to outline the course of evolutionary development within the genus *Pleospora*. The present paper is the first of several which will attempt to analyze more closely this situation; to group the collections studied into workable species, and attempt to find the proper binomials for these groups.   The collections considered here are the simpler types which illustrate the origin of the main lines of development within the genus.

In the earlier paper mentioned it was shown that if a large number of collections having, for instance, a similar spore form were arranged according to spore size, a long series with overlapping spore measurements is obtained, often extending over the range of many described species.   This same situation occurs as regards almost any pair of unit characters of spore form, size, septation, color, etc.   Although there seem to be an infinite number of such combinations and variations, there is a certain correlation which allows a pattern of development to be discerned. Three such general lines of development, based on spore form and septation, were outlined, and for convenience in the following discussion the characters distinguishing each are briefly listed below.

Leptosphaeroid series:
 spores fusoid, strongly tapered at the ends;
 spores yellow-brown to dark yellow-brown;
 secondary septa laid down as transverse walls in the end cells; vertical septa tardily formed.
Vulgaris series:
 spores ellipsoid, ends tending to be more bluntly rounded;
 spores often inequilateral to curved;
 spores yellow-brown to red-brown;
 secondary septa laid down as both transverse and vertical walls, more or less simultaneously in the central cells, end cells normally without vertical septa.

Herbarum series:

> spores oblong-ellipsoid, ends broadly rounded or bluntly tapered;
> spores more often straight, either symmetric or asymmetric;
> secondary septa as in vulgaris series but with additional, vertical, or "Y" shaped septa in the end cells;
> spores yellow- to red-brown, more commonly red-brown.

The collections discussed in this paper are referred to by numbers, which are those of the writer's files. A list, in numerical order, with the data for each, is given at the end of the paper.

It should be definitely understood that the delimitation of species, from such a pattern as mentioned, is of necessity an arbitrary one and based upon personal judgment, but such a delimitation is necessary in order to have a basis for future revisions.

The basic spore form of the genus is a three-septate one. These first three septa are referred to as the primary septa. The origin of all the main lines of variation is foreshadowed within this group. The septation of the three-septate spore is, of necessity, leptosphaeroid, but the form and color changes of other series are found. *P. pellita* (FIG. 6) has the typical straight, tapered, sometimes asymmetric, yellow-brown spore of the leptosphaeroid series. *P. trichostoma* (FIG. 1) and *P. mollis* (FIG. 2) represent a small unrelated group, with oblong spores. In *P. oligostachyae* (FIG. 9) and *P. diaporthoides* (FIG. 10) the inequilateral or curved form of the spore is more pronounced and the ends are more bluntly tapered. These are tendencies leading to the vulgaris series. In the broader spores of this type vertical walls are occasionally seen in the end cells even in three-septate spores, indicating an early origin of this herbarum-series characteristic. It is in such broad spores [*i.e., P. Boldoae* (FIG. 12), *P. lactucicola* (FIG. 13)] also in which the vulgaris type of septation of the central cells first appears. Certain species with three-septate spores also show tomentose or setose perithecia, and specimens on woody substrata show the elongate asci, uniseriate spores and large perithecia characteristic of this habitat.

PLEOSPORA TRICHOSTOMA AND P. MOLLIS

The first two species considered belong to an unrelated group which does not fit into any of the three series mentioned because

of the cylindric-ellipsoid spores with almost straight side walls and rounded ends. *P. trichostoma* has large light colored ascospores and large stromatic perithecia which are often setose. It is fairly common on stems of grasses. *P. mollis* which has smaller dark red-brown spores and small perithecia is based on a single collection, on *Ephedra,* from the Andes. ·

PLEOSPORA TRICHOSTOMA (Fr.) Ces. & de Not.   Schema sfer. ital. 217.   1861.

Sphaeria relicina Fr. Syst. Myc. 2: 505.   1823.

Sphaeria trichostoma Fr. Syst. Myc. 2: 504.   1823.

Sphaeria polytricha Wallr. Fl. Crypt. Germ. 794.   1833?.

Pyrenophora trichostoma (Fr.) Fck. Symb. Myc. 215.   1870.

Pleospora relicina (Fr.) Wint. in Rab., Krypt. Fl. 1 (2): 520. 1887.

Pleospora polytricha (Wallr.) Tul. Sel. Fung. Carp. 2: 268.   1863.

Pyrenophora relicina (Fr.) Fck. Symb. Myc. 215.   1870.

Sphaeria culmorum Cke. Grev. 3: 68.   1874.

Pleospora culmorum (Cke.) Sacc. Syll. Fung. 2: 263.   1883.

Pleospora Bromi Died. Centralbl. f. Bakt. Abt. 2, 11: 52.   1903.

Pleospora teres Died. *Ibid.*   1903.

Pleospora Tritici-repentis Died. *Ibid.*   1903.

Pleospora Avenae Died. *Ibid.*   1903.

Pleospora graminea Died. *Ibid.*   1903.

Pleospora trichostoma f. sp. *Bromi* (Died.) Noack Zeitschr. Pflanzenkr. 15: 193.   1905.

Pleospora trichostoma f. sp. *Tritici-repentis* (Died.) Noack *Ibid.* 1905.

Pleospora trichostoma f. sp. *Hordei erecti* Noack *Ibid.*   1905.

Pyrenophora Teres (Died.) Drechsler Journ. Agr. Res. 24: 656. 1923.

Pyrenophora Tritici-repentis (Died.) Drechsler *Ibid.* 667.   1923.

Pyrenophora Bromi (Died.) Drechsler *Ibid.* 672.   1923.

Illustration.   Figure 1.

Perithecia 300–500(750) × 200–350 μ, sclerotioid, usually rather thickly scattered, somewhat flattened-spheric, strongly erumpent, often becoming superficial, ostiole indefinite or flattened-papillate,

with a few or many, shorter or longer, stiff, brown, septate, pointed setae about the ostiole or the upper portion of the perithecium (sometimes broken off or wanting), wall thick-stromatic, with an outer blackened layer 10–20 μ thick and an inner hyaline parenchymatic layer 50–100 μ thick.

Asci stout clavate, with a much thickened wall, especially at the apex, base claw-like, 160–210 × 35–50 μ, with some interthecial tissue.

Spores biseriate, oblong-ellipsoid, 3-septate, rather light olive-brown, ends broadly rounded, constricted at all septa, often with a vertical septum in one or two cells, 44–60 × 18–25 μ.

Collections: *449, 450, 451,* on *Poa, Secale* and *Triticum,* from Europe.

The collections grouped here represent a species complex which is very characteristic. It is found on the culms of grasses and has large sclerotioid perithecia with stiff apical spines. These spines are most abundant on sterile stromata and probably represent conidiophores which may bear the conidial stage, but may be broken off or be entirely lacking in some cases. The spores are characteristic, being large, oblong-ellipsoid and three-septate.

This species has been studied rather extensively by various plant pathologists. Diedicke (3) studied strains from different cereals and found conidial stages belonging to *Helminthosporium* spp., and differences in ability of different strains to infect different host genera. On perhaps incorrect evidence he excluded from *P. trichostoma* all races which produced such *Helminthosporium* stages and erected five new binomials (*Pleospora teres, P. Bromi, P. Tritici-repentis, P. Avenae* and *P. graminea*). These were to apply to the ascus stages of *Helminthosporium* species occurring on different host genera and were purely hypothetical in some cases. No specific descriptions were given. Noack (7) later considered these species as physiologic forms, which is a more logical treatment. Drechsler (4) recognized three of these species and transferred them to the genus *Pyrenophora* (*P. teres, P. Tritici-repentis,* and *P. Bromi*).

There is a great deal of variation in the size of the perithecium, ascus and ascospore within individual collections of this species group, and there may be some correlation between these characters

and the host occurrence, which would be a basis for varietal separation, but so far no such evidence has been seen.

The synonymy of the binomials given above is very much interlocked in the literature.   These species all have the same large
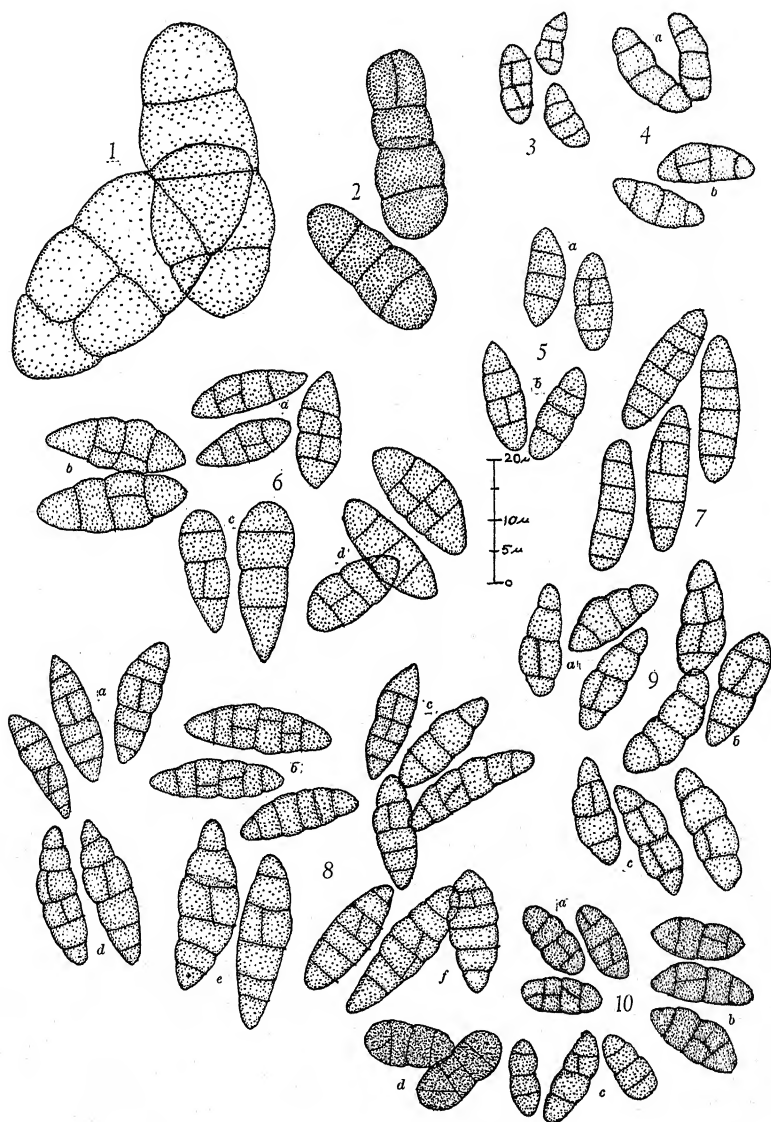
FIGS. 1–10. Spores of *Pleospora*.

3-septate ascospores (see Berlese 1, vol. 2, *pl.* 51). Berlese (1, vol. 2: 34) also gives *Pleospora sarcocystis* (B. & C.) Sacc. as a synonym. He distinguishes *Pleospora culmorum* (Cke.) Sacc. only on the lack of setae, which is not dependable. His figures of *Pleospora typhicola* (Cke.) Sacc. (1, vol. 2, *pl.* 9, *fig.* 2) show vertical septa occurring more commonly in the end cells of the spores, but otherwise it is very similar to *P. culmorum.*

PLEOSPORA MOLLIS Starb., Arkiv. f. Bot. **5**: 24. 1905.

Illustration. Figure 2.

Perithecia small, 100–150 μ in diameter, somewhat flattened-spheric, thickly scattered in small seriate groups, immersed in the stem and erumpent in seriate lines of small longitudinal ruptures of the epidermis; wall 10–30 μ in thickness, of dark black, coarse parenchyma.

Asci few, stout-saccate, expanding rapidly when freed due to a gelatinous substance about the spores, and soon disappearing, 35–70 × 18–26 μ.

Spores 2- to 3-seriate, oblong, cylindric, dark red-brown, 3-septate, straight, ends bluntly rounded or with a slight taper, becoming constricted at the septa at maturity, vertical walls appearing in one or sometimes two cells (either central or end cells), 23–26.5 × 7–9 μ.

Collection: *35,* on *Ephedra,* from the Argentine Andes (*Type*).

This species is unique in the possession of dark, red-brown, 3-septate, cylindric spores (FIG. 2).

PLEOSPHAERIA HYPHASMATIS AND PYRENOPHORA DELICATULA

These two species would be excluded by many from the genus *Pleospora* because of the superficial position of the perithecia. They show, however, a primitive type and the origin of the dictyo-spore from the phragmospore type, for it is sometimes difficult to find spores with a vertical septum in any of their cells. In the tomentose or setose character of the perithecium they are similar to *Pleospora calvescens.*

PLEOSPHAERIA HYPHASMATIS (E. & E.) Berl., Icon. Fung. **2**: 63. 1900.

Pyrenophora hyphasmatis E. & E. Journ. Myc. **4**: 77. 1888.

Illustration.   Figure 3.

Perithecia scattered, superficial, 250–400 μ in diameter, globose, with a conic to cylindric, prominent ostiole, covered with a tomentum of fine, soft, matted, somewhat septate, brown hairs, often collapsing in age.

Asci clavate, becoming more elongate at maturity, 60–80 × 8–9 μ, base claw-like, imbedded in an agglutinate mass of interthecial tissue.

Spores, biseriate, becoming overlapping uniseriate, broadly fusoid-ellipsoid, 3-septate, yellow-brown, straight or inequilateral, mostly symmetric, slightly constricted at the septa, ends acute or slightly rounded, often with a vertical septum in one of the central cells, 9–12.5 × 5.5–6.5 μ.

Collection: *1 (Type)*, on piece of rag, Louisiana.

This species may be considered as one of the most primitive of the genus. The spores (FIG. 3) are small, 3-septate and show vertical septa in less than fifty per cent of the individual spores. The perithecia are superficial and densely tomentose, but not setose. It also illustrates nicely the convergence of many described genera. Many such collections with only a few spores showing vertical septa have no doubt been described under *Leptosphaeria*. Because of the hairy perithecia it might be found in *Pocosphaeria*, or because of their superficial position, under *Venturiella*. Because of the vertical septa and hairy perithecia, Ellis placed the collection in *Pyrenophora*, even though it is not setose. Because of the superficial position, Berlese placed it in *Pleosphaeria*. If one disregards the hairiness as a generic character, it would fall in *Teichospora*.

PYRENOPHORA DELICATULA Vestergr., Jahreskatalog d. Wien. Krypt. Tauschanstalt **1897**: 3.

Illustration.   Figure 4.

Perithecia minute, 70–100(150) μ in diameter, imbedded in the dense tomentum of the leaves, globose to conic; ostiole conic, surrounded by a fascicle of short, stout, stiff, pointed, brown, spine-like hairs 15–30(60) × 3–3.5 μ; wall thin membranous.

Asci clavate, wall only slightly thickened, 47–55(70) × 12.5–14 μ.

Spores biseriate, fusoid-ellipsoid with ends somewhat rounded, 3-septate, pale yellow-brown, inequilateral, one side flattened, or

somewhat allantoid, not constricted or very slightly so, most spores without vertical septa, but some spores with vertical septa in one or two central cells, 14.5–18 × 6–7 μ.

Collections: 2 (*Cotypes*), *60;* on *Cerastium,* from Sweden.

These perithecia have a definite fascicle of setae about the ostiole as given for *Pyrenophora.* They are imbedded in the leaf hairs, but are actually superficial. The spores (FIG. 4) are larger and more curved that in *P. hyphasmatis.* It might again be placed in *Pleosphaeria. P. calvescens* (FIG. 5) differs in the straighter spores and the larger immersed perithecia which are both tomentose and setose.

PLEOSPORA CALVESCENS AND P. PELLITA

There is some confusion in the literature concerning these two binomials. The spores are very similar, being 3-septate with occasional vertical septa in the central cells (FIGS. 5, 6), but two distinct species groups are discernible. The descriptions of Tulasne as well as most subsequent usage place *P. calvescens* as a form having perithecia with both a dense tomentum and stiff setose hairs and occurring on *Atriplex, Chenopodium,* etc. The spores of this species tend to be slightly inequilateral and somewhat smaller than those of *P. pellita* which has perithecia which are smooth or with a few soft hairs (conidiophores) and occurs mostly on *Papaver.* Berlese (1, vol. 2: 6) unites these two species, but his *Pyr. echinella* (1, vol. 2: 33) is probably the true *P. calvescens.*

PLEOSPORA CALVESCENS (Fr.) Tul., Sel. Fung. Carp. 2: 266. 1863.

Sphaeria calvescens Fr. in Scler. Suec. 401.
Sphaeria Dematium (Pers.) Klotzsch in Herb. Myc. 154. 1832.
Cucurbitaria calvescens (Fr.) de Not. Schema Sfer. ital. 215. 1861.
Pyrenophora calvescens (Fr.) Sacc. Syll. Fung. 2: 279. 1883.

Illustration. Figure 5.

Perithecia 200–300 μ in diameter, globose to somewhat depressed or flattened, thickly scattered or crowded, soon erumpent,

covered with a velvety tomentum of stiff brown hyphae which
become straight, erect and setose on the upper surface of the peri-
thecium, $50–100 \times 5–7\,\mu$.   Perithecia stromatic, walls $20–30\,\mu$
thick, of dark brown parenchyma.

Asci cylindric-clavate, with thickened apical walls and claw-like
base, $70–95 \times 8.5–12\,\mu$, imbedded in a rather abundant interthecial
tissue.

Spores biseriate to overlapping uniseriate, fusoid-ellipsoid, yel-
low-brown, 3-septate, with pointed to slightly rounded ends, sym-
metric but often somewhat inequilateral, vertical walls in one or
two of the central cells in a small per cent of the spores, $12.5–19$
$\times 5.5–7\,\mu$.

Collections: *3, 4, 5;* on *Atriplex* and *Chenopodium,* Germany.

Both de Notaris and Tulasne base their species on Fries, Scler.
Suec. No. 401, which has not been seen, but Tulasne's description
and most collections show the tomentose-setose perithecia which
are characteristic.   These stiff hairs may fall off or be worn away,
and then the perithecia appear smooth and shiny.

PLEOSPORA PELLITA (Fr.) Rab., in Herb. Myc. II, No. 749.
1858.

Sphaeria pellita Fr. Syst. Myc. 2: 502. 1823.
Sphaeria Papaveris Tul. Sel. Fung. Carp. 1: 45 & 227. 1861.
Cucurbitaria papaveracea de Not. Sfer. ital. 62. 1863.
Pyrenophora pellita (Fr.) Sacc. Syll. Fung. 2: 280. 1883.
Pleospora papaveracea (de Not.) Sacc. Syll. Fung. 2: 243. 1883.

Illustration.   Figure 6.

Perithecia $200–300(400)\,\mu$ in diameter, globose or somewhat
flattened, sometimes collapsing, thickly scattered, or grouped in
small seriate clusters which are erumpent through a longitudinal
slit in the epidermis; walls rather thick, $20–30(50)\,\mu$, parenchy-
matic, often lighter colored below; smooth or with a few scattered
setose conidiophores which soon break off, leaving short stubs.

Asci clavate to cylindric-clavate, wall somewhat thickened, base
claw-like, $70–105 \times (8.5)10–14\,\mu$, imbedded in rather numerous
interthecial strips.

Spores biseriate to overlapping uniseriate, fusoid or slightly
clavate, pale yellow-brown to yellow-brown, 3-septate, usually
straight, or slightly inequilateral, symmetric or often asymmetric
with a longer more tapered lower portion, somewhat constricted

at the septa, with vertical septa in one or two central cells in most spores, ends rather acutely tapered, $(14)16–21(26) \times 6–8\ \mu$.

Collections: *7, 9, 10, 11, 12, 13, 14, 120a, 352, 424,* on *Papaver, Salsola,* and *Bardana,* from Central Europe and Italy, and N. Dakota in the United States.

Cesati and Notaris (2, p. 218) and Tulasne (8, vol. 2: 268) both cite Rab. Herb. Myc., Ed. II, 749 (No. *12*) as a basis of this species. This exsiccatus is typical of *P. pellita* as given above, showing the stubs of conidiophores, which have been broken off, on the perithecia. Tulasne comments that the *Sphaeria pellita* of Fries may not be the same fungus.

This species may be considered as a starting point for the series with smooth perithecia. The spores (FIG. 6) are more commonly found with vertical septa than in the species with hairy or setose perithecia just described, and they are usually straight (not inequilateral), but show a tendency to be more or less elongate and tapered below. The various collections (see table) show a good deal of variation in size but not enough for further separations. Most of the collections are on *Papaver,* and *Pleospora papaveracea* is probably a synonym.

No. *424,* of *P. Bardanae* Niessl (FIG. 6b), on *Althaea,* has large asymmetric spores, but No. *14* (FIG. 6c), on *Papaver,* has even more clavate spores of about the same size. Niessl's (6: 178) description of *P. Bardanae* agrees well with *P. pellita* except that he gives the spores as rounded above, tapered below and inequilateral or curved, which suggests the complex of collections placed in *P. diaporthoides.* Berlese (1, vol. 2, *pl. 6, fig. 1*) figures spores for *P. Bardanae* which are similar to those of *P. pellita,* but, again, slightly curved.

There are two fungi on collection No. *120,* of *P. Lecanora* Fabre, on *Salsola* from N. America. No. *120a* (FIG. 6d), with 3-septate spores, most resembles Fabre's description. These spores are shorter, broader and with more rounded ends than those typical of *P. pellita.* Vertical septa are very rare in these spores. This single collection of rather sparse material is not sufficient for a description, however, and other collections are needed. These spores have the form of those of *P. diaporthoides,* but are longer, and a paler yellow-brown.

THE PLEOSPORA VAGANS COMPLEX

P. calvescens and *P. pellita* have the simplest type of lepto-sphaeroid spores. The following species, *P. vagans,* as here conceived, includes a rather compact group of collections occurring on grasses, and has spores which can be derived from the *P. pellita* type by the insertion of an additional transverse septum in each of the end cells, giving a five-septate spore.

TABLE*

Coll. No.	Host	Spores	Asci	Perithecia
		Pleospora trichostoma (Fr.) Ces. & De Not.		
450	*Poa*	44–46 ×18–20	180–210 ×40–45	350–400, S, D
449	*Secale*	44–53 ×18–21	175–200 ×35–44	400–500, S, D
451	*Triticum*	44–60 ×18–25	160–200 ×40–50	300–400, D
		Pleospora calvescens (Fr.) Tul.		
3	*Chenopodium*	12.5–16 ×5.5–7	78–95 ×8–9	200–250, S, T
4	*Atriplex*	13–16 ×5.5–5.5	70–78 ×8.5–10	200–300, S, T
5	*Chenopodium*	13.5–19 ×5.5–6.5	75–85 ×10–13	200–300, S, T
		Pleospora pellita (Fr.) Rab.		
7	*Papaver*	14–19 ×6–7	78–90 ×10–12	200–300
352	*Papaver*	16–18 ×6–7	70–78 ×12.5–14	250–300
10	*Papaver*	16–19.5 ×6–7	75–95 ×10–12	250–350, c
9	*Papaver*	16–19.5 ×6–7		200–250
11	*Papaver*	16–20 ×6–7	78–90 ×11–13	200–250, D, C
120a	*Salsola*	17–21 ×7.5–8	85–110 ×12.5–14	200–300, D, C
14	*Papaver*	17–23 ×6–7		200–300, S
12	Herb	18–21 ×6–7	78–95 ×8.5–11	200–300, d, c
13	*Papaver*	18–21.5 ×6–7.5	78–95 ×8.5–11	300–400
424	*Bardana*	20–26 ×7–8	88–106 ×12.5–13	250–300
		Pleospora vagans Niessl		
23	*Clematis*	16–23 ×5.5–7	85–105 ×10–12	300–400
24	*Aira*	17–21.5 ×5.5–7	60–75 ×10.5–12.5	200–300, D
339	*Poa*	18–21.5 ×6–7	60–70 ×14–16	150–200, d
25	*Phleum*	18–22 ×5.5–6	60–65 ×13–15	150–250, d
26	*Phleum*	18–23 ×6–7	65–70 ×14–15	150–200
27	*Poa*	18–23 ×6–7	60–70 ×12–13	150–200
332	*Poa*	19–21.5 ×6–7	60–70 ×14	150–200
28	*Melica*	19.5–22.5 ×6–7	62–70 ×16–17	150–300, D
29	*Melica*	19.5–23 ×7	70–78 ×16–18	150–250
30	*Festuca*	20–23.5 ×7–8	70–90 ×17–18	250–350, d
335	*Agrostis*	20–25 ×8–9	70–78 ×19–21	300, D
324	*Calamagrostis*	21.5–23 ×7.5–8.5	60–80 ×14–17	250–350, D
325	*Elymus*	21.5–26.5 ×8.5–9.5	70–80 ×17–19	200–250, D
32	*Poa*	22–26 ×6–7	80–85 ×16–18	150–200
329	*Calamagrostis*	23–25 ×7–8	70–75 ×15–16	250–350
34	*Festuca*	23–26 (30) ×8.5–9	70–90 ×14–18	200–250, D

* This table gives data for the individual collections.   The letters T, S, D, and C stand for tomentose, setose, depressed and collapsing, respectively. Small letters indicate a lesser degree of the same condition.

PLEOSPORA VAGANS Niessl, Verhandl. naturf. Ver. in Brünn 14: 174. 1876.

Pleospora fuegiana Speg. Fung. Fueg. (Bol. Acad. Nac. Cienc. de Cordoba 11: 135). 1887.

Pleospora Forsteri Speg. Fung. Fueg. *Ibid.*

Illustration.   Figure 8.

Perithecia 150–350(400) μ, mostly flattened-spheric, immersed beneath the epidermis, appearing on the surface as thickly scattered, separately erumpent papillate ostioles, each often surrounded by a minute circular grayish spot where the perithecia show through the epidermis, at other times forming small seriate or confluent clusters, causing elongate ruptures of the epidermis; walls rather thin (5–15 μ) membranous, parenchymatic, smooth.

Asci clavate, walls somewhat thickened, especially at the apex, base claw-like, 60–90 × 11–18(21) μ.

Spores biseriate, fusoid, yellow-brown to dark yellow-brown, 5-septate, straight, inequilateral or slightly curved, symmetric or slightly asymmetric due to the slight inflation of the third cell, vertical walls appearing in one or two central cells, slightly constricted or not constricted at the septa, the two central cells usually slightly larger than the other cells, end tapered, 17–26(30) × 5.5–9 μ.

Collections: *23, 24, 25, 26, 27, 28, 29, 30, 32, 34, 324, 325, 329, 332, 335, 339,* on various grasses, from Scandinavia and Tierra del Fuego.

Var. PUSILLA Niessl: l.c.

As described above except spores rather narrow, more tapered at the ends and more constricted at the septa, 17–23(26) × 5.5–7(8) μ.

Collections: *24, 25, 26, 27, 28, 29, 30, 32, 329, 332, 339.*

Var. ARENARIA Niessl: l.c.

As in the typical variety but spores larger, particularly broader, less strongly tapered, and less strongly or not at all constricted at the septa, 20–26(30) × 7.5–9.5 μ.

Collections: *34, 324, 325, 335.*

This group of collections on grasses forms a rather distinct species, which is no doubt the *Pleospora vagans* of Niessl. Most of the collections seen have come from Northern Europe, but it is

interesting to see several Spegazzini specimens from the southern tip of South America with almost identical characters.

Niessl described three varieties as follows:

a. *arenaria:* with perithecia on discolored spots, 250–270 μ; asci 105–120 × 21–23 μ and spores straight, clavate-fusoid, 27–30 × 9–10 μ. The elongate asci suggest *P. findens,* but the broad clavate spores do not; it is probably merely a well-matured condition in which the asci are stretching for ejection of spores.

b. *pusilla:* with perithecia small, 150–180 μ, not in discolored spots; asci 60–80 × 18–20 μ; spores 22–24 × 8–9 μ, with third cell swollen.

c. *Airae:* with clustered or seriate perithecia 220–250 μ in diameter; asci 75–90 × 16–18 μ and spores 21–26 × 8 μ.

There is some variation within the species (see table). There is one group of collections which seem to have broader, less strongly tapered spores, with slighter constrictions. These are placed in the variety *arenaria.* Niessl's variety *pusilla* is most typical of the species and is retained for the narrower spore type. Intergrades are, of course, found between these two varieties.

Berlese (1, vol. 2: 9) gives the spores of the variety *pusilla* as larger than those of *Airae,* but his figures (1, vol. 2, *pl.* 11, *figs.* 2 & 3) are both typical of the species as here described. His figures of spores (1, vol. 2, *pl.* 12, *fig.* 1) of the var. *Sparganii* Cke. are broader and characteristic of the var. *arenaria,* as here delimited. Berlese's figures (1, vol. 2, *pl.* 12, *figs.* 2 & 3) of *P. donacina* Niessl and *P. microspora* Niessl are also similar to this species, but Niessl gives the former as having larger, thick-walled, greenish spores, often with several vertical septa in each cell, and the latter as 3- sometimes 5-septate. Berlese's measurements for the spores of *P. microspora* are only 18 × 8 μ.

The type of *Pleospora fuegiana* Speg. (*32*) shows scattered small (150–200 μ) perithecia in the leaf tissue of *Poa Forsteri* erumpent as minute ostioles. The asci are clavate and 80–85 × 16–18 μ. The spores (FIG. 8d) are typical, in form, of the species, and 22–26 × 6–7 μ. They are rather long, but have the narrow tapered form of the var. *pusilla.*

The type collection of *Pleospora Forsteri* Speg. (*34*) shows the somewhat elongate type of pustule on stems of *Festuca magel-*

lanica. The perithecia are flattened and $200–250 \times 100–150\,\mu$. The asci are $70–90 \times 14–18\,\mu$ and the spores (FIG. 8e) are $23–26(30) \times 8–9.5\,\mu$. The material is rather poor, but it fits Spegazzini's description and the broad spores are characteristic of the variety *arenaria.*

The spores of *P. vagans* differ from those of *P. pellita* not only in the addition of an extra septum, but also in the more symmetric but somewhat inequilateral form. The insertion of the extra transverse septa in the end cells causes the two central cells to be somewhat larger and the upper (third) cell is often somewhat swollen, as is commonly found in spores of *Leptosphaeria.* Collection *23,* of *P. parvula,* is one of those difficult ones which is intermediate between several species. Some perithecia show mostly 3-septate spores, others show many spores with one or two secondary septa laid down in the end cells giving 4- or 5-septate spores, whereas others show mostly 5-septate spores. These spores (FIG. 8c) are transitional between *P. pellita, P. vagans* and those with asymmetrical septation. The perithecia of this collection have the thick stromatic walls of *P. pellita* but the spores are larger and more like *P. vagans.* It is collections such as this that suggest that hybridization or perhaps heterokaryosis may occur and give rise to spores of two similar types. Such collections must be placed in a purely arbitrary fashion, as this one is in *P. vagans.*

The spores of *P. parvula,* as figured by Berlese (**1,** vol. 2 : 5; *pl.* 5, *fig.* 1), suggest the shorter, stouter spores of *P. calvescens.* The long, narrow, cylindric asci and large perithecia of this collection are also correlated with its occurrence on woody stems, and it may eventually have to be segregated as a separate species.

PLEOSPORA FINDENS E. & E., Amer. Nat. **31** : 342. 1897.

Illustration. Figure 7.

Perithecia $350–400\,\mu$ in diameter, globose, rather deeply seated in the culm and erumpent as separate or somewhat linear clusters of conic ostioles; wall $10–20\,\mu$ thick, of rather dense, black-brown, parenchyma.

Asci long-cylindric, apical wall slightly thickened, base claw-like, $140–160 \times 10–12\,\mu$, rather numerous, interspersed by numerous interthecial strips.

Spores uniseriate to overlapping, oblong-ellipsoid to almost cylindric, yellow-brown, 5-septate, mostly straight, symmetric, ends bluntly rounded, vertical walls rather uncommon but found in one or two of the central cells of a number of the spores, constrictions none or very slight, 21.5–24.5 × 6–7 μ.

Collections: *31 (Type)*, on *Andropogon,* from New Jersey.

The above description is taken from the type collection of *P. findens*. It differs from *P. vagans* in the straight spores which have more nearly parallel side walls and bluntly rounded ends, giving a more cylindric form. The asci are also longer and narrower, with uniseriate spores, and the perithecia are larger and contain more interthecial strips (pseudoparaphyses). In these latter characters it resembles the species of *Pleospora* on woody stems, and in fact the spores of this species are very similar to those of *P. atromaculans* on *Cornus* and might be derived from them by the addition of secondary septa in the end cells.

PLEOSPORA OLIGOSTACHYAE AND P. DIAPORTHOIDES

It is necessary to consider at this point a rather confusing group of collections, which show the changes in spore form and septation which lead to the vulgaris series at the 3-septate level. The spores of some of these collections are very similar to and difficult to distinguish from those of *P. pellita*. In some cases it is necessary to place collections in a purely arbitrary manner. The general difference of the spores of this group (FIGS. 9–13) is that they are somewhat broader in appearance due to the lesser taper toward the ends, and tend to be inequilateral or curved, but symmetric, rather than straight and asymmetric, as in the *pellita* type. The occasional appearance of secondary septa, of the *vulgaris* type, in the central cells (see **10**), in these collections, gives rise to spores typical of the vulgaris series. It has also been observed that vertical walls are not uncommonly found in the end cells of spores which are only 3-septate in some collections. Such septation indicates an early tendency toward the herbarum series.

The binomial *Pleospora oligostachyae* is used for the first species of this group. It is represented by several collections on grasses, which have spores similar to those of *P. pellita* but more inequilateral-ellipsoid in form.

PLEOSPORA OLIGOSTACHYAE E. & E., Amer. Nat. **31** : 342.   1897.

Illustration.   Figure 9.

Perithecia 150–250(300) μ in diameter, thickly scattered, or sometimes seriately arranged, globose or slightly flattened, immersed in the culms, with small papillate erumpent ostioles; walls rather thin, 10–20 μ thick, of coarse, light brown parenchyma.

Asci clavate to cylindric-clavate, with a claw-like base and a somewhat thickened wall, 70–88 × 10–12.5 μ.

Spores biseriate, fusoid-ellipsoid, yellow-brown to dark yellow-brown, 3-septate, mostly somewhat inequilateral or slightly curved, constricted at the central septum, sometimes at the secondary septa, ends somewhat tapered but rather bluntly rounded, rarely somewhat asymmetric, usually with vertical septa in one or two of the central cells, never in the end cells, 16–20 × 5.5–7 μ.

Collections : *8, 17 (Type), 61a,* on *Sporobolus, Bouteloa* and *Andropogon,* from Kansas.

The type collection of *P. oligostachyae* is typical of this group of collections on grasses.   The spores (FIG. 9c) are perhaps closest to those of *P. pellita* of this intermediate 3-septate group of species between the leptosphaeroid and vulgaris series.   They differ from those placed under the following species in being larger, more tapered and more inequilateral, and they never show vertical walls in the end cells.   *Pleospora Andropogonis* Niessl may be this same fungus, but the asci (90–96 × 24–27 μ) and spores (18–21 × 10–12 μ), as given by Niessl, are definitely broader than in *P. oligostachyae.*

Pleospora hysterioides E. & E., Erythea **2** : 19. 1894. The type collection *(61)* of this species bears two fungi with muriform spores, which apparently were confused by Ellis.   One of these is of the *Hysteriographium* type with elongate (300–600 × 60–100 μ) flat ascocarps with carbonaceous walls 10–18 μ thick, and with a longitudinal ostiolar slit.   The ascospores of this fungus are clathrate, flattened, narrow ellipsoid, rather dark yellow-brown, 3-septate, with vertical septa in the two central cells in face view, but showing no vertical walls in edge view. They are 13–18 × 5.5–7.5 × 3.5–4.5 μ.   This same fungus was apparently described by Ellis (**5** : 53) in 1900, as *Hysteriographium graminis* E. & E.   Ellis' spore measurements (15–20 ×

7–10 × 5–8 μ), in both cases, were somewhat larger than those obtained by the writer. Ellis' description of the *Pleospora* indicates that he was referring to this fungus, which must therefore be the type. The proper binomial, therefore, is **Hysteriographium hysterioides** (E. & E.) comb. nov.

This species may belong in Petrak's genus *Pseudopleospora,* which is given as having elongate perithecia opening by a slit, but the spores of his *P. ruthenica* are given as pyriform and no mention is made of any clathrate character.

The second fungus (*61a*) on the type collection is a good *Pleospora* with globose perithecia and three-septate spores (9b) like those of *P. oligostachyae.*

Ellis saw both fungi but apparently thought they were the same, for on a second packet (*8*) containing a collection on *Sporobolus,* made five days later, he says, "Spec. with perithecia orbicular and opening above with a single round pore. Found with others having a longitudinal dehiscence." On the paper of the first packet he has drawings of the *Pleospora* spores, but on an enclosed slip there are drawings of the *Hysteriographium* spores.

The following species includes a somewhat more heterogeneous group of collections, on hosts other than grasses, and with somewhat smaller spores (FIG. 10) which are more rounded at the ends, not inequilateral, and often showing vertical septa in the end cells. These spores are more of the type of the vulgaris series or even the herbarum series.

PLEOSPORA DIAPORTHOIDES E. & E., Proc. Acad. Sci. Phila. 1890 : 238.

Pleospora cereicola Speg. Fung. Chil. 85. 1910.

Illustrations. Figures 10–11.

Perithecia 150–350 μ in diameter, scattered, globose, ostiole papillate, or somewhat elongate, walls 10–20 μ thick, parenchymatic.

Asci clavate to cylindric, base claw-like, wall somewhat thickened, 70–97 × 7–12.5 μ.

Spores biseriate to overlapping uniseriate, ellipsoid, yellow-brown to red-brown, 3-septate, mostly straight or very slightly

inequilateral, mostly symmetric or somewhat tapered below, ends mostly rather broad and blunt, occasionally tapered, vertical walls in one or both central cells and occasionally in the end cells (12.5)14–18 × (4.5)5.5–7 μ.

Collections: *6, 15 (Type), 423, 429,* on *Bardana, Pastinaca, Cereus* and *Tillandsia,* from California, New Jersey and Argentina.
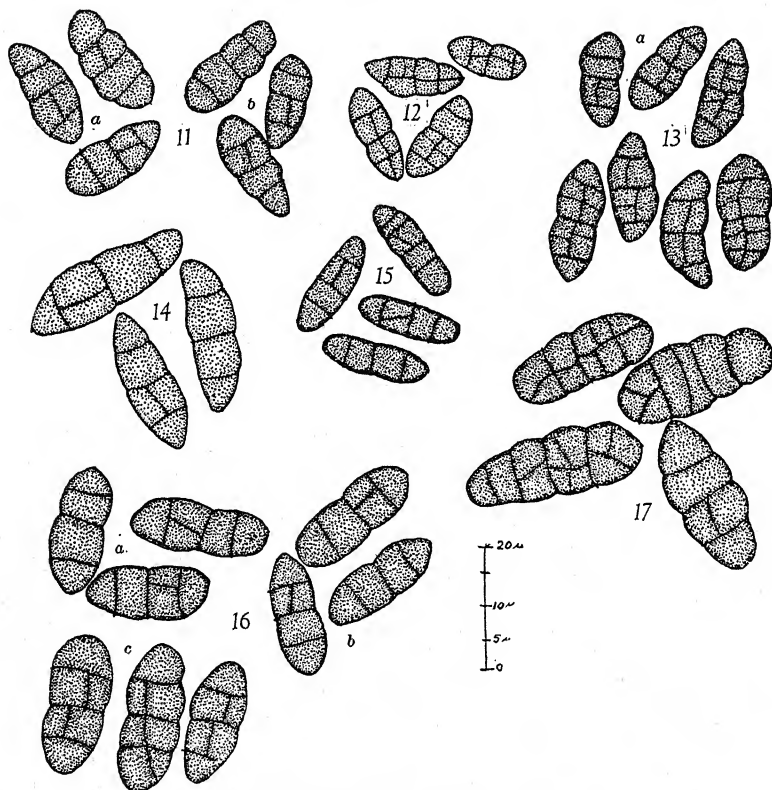
Figs. 11–17. Spores of *Pleospora.*

This is a rather heterogeneous group of collections, but they all show a common spore type which is straighter and with more blunt ends than in either *P. oligostachyae* or *P. pellita* (figs. 10, 11) and vertical walls occasionally appear in the end cells.

The type collection (*15*) of *P. diaporthoides* shows dark red-brown spores (fig. 10b) which occasionally show faint secondary

septa of the *vulgaris* type in the central cells.   A second collection
(*16*) of this species, which has rather dark brown spores, shows
such secondary septa in many of its spores and it is transferred
to *P. lactucicola* E. & E.   In the Riksmuseet, there is a packet
(*6*) labelled *P. diaporthoides* E. & E., *Bardana, N. J.,* but it also
bears a pasted label "Pl.S.Calif. 5384, of *P. lactucicola* E. & E.,
on *Coleosanthus californicus.*"   The same collection appears under
the same number in the New York Botanical Garden, with the
comment "det. Rehm or *P. lactucicola* plus *Lophiostoma insidio-
sum.*"   The New York packet yielded nothing but a *Lepto-
sphaeria,* but the one in the Riksmuseet bears a *Pleospora* with
spores (FIG. 10a) as here described but rather small (12.5–14 ×
5.5–6 μ) and yellow-brown.

The type collection of *Pleospora cereicola* Speg. (*423*) is very
poor material, but it yielded a few perithecia with spores (FIG.
10d) of the *P. diaporthoides* type, dark brown and with occasional
vertical septa in the end cells.   It is placed here as a synonym
for the present.

The type collection of *Leptosphaeria aerea* Speg. (*429*) yielded
only a few perithecia with spores.   Berlese (**1**, vol. 1 : 87) says
this type collection is sterile.   Many of these spores (FIG. 10c)
showed vertical septa in some of the cells, and, in fact, Spegazzini
shows such vertical septa in his figures on the packet.   This
should be a *Pleospora.*   These spores are of the general type here
considered, but are rather small (12.5–16 × 4.5–5.5 μ) and yellow-
brown, in which respects they agree with (*6*).   These two collec-
tions might be considered a variety or species, but too few collec-
tions are yet available for such a segregation.   The spores of *L.
aerea* are also more asymmetric and tapered below than the other
collections of this group.

Two collections (Nos. *447* & *492*) recently examined from
India, on *Zizyphus* and *Sueda,* show spores (FIG. 11) similar to
those of this species, but somewhat broader and more rounded at
the ends.   The perithecia are also early and strongly erumpent,
resembling a *Teichospora* at maturity.   They approach *P. carpini-
cola,* on woody stems, and may represent a separate species if
confirmed by more collections.

Pleospora clypeata sp. nov. (FIG. 14)

Perithecia 300–500 μ diametro, aspectu superficiali sicut maculae rotundae, atrae, dispersae, in cortice immersa, dein erumpentia ut ostiola brevia cylindrica; pariete 50 μ crasso, ex parenchymate crasso, atro constituto; clypeo stromatico rotundato, 100 μ crasso praedita, ex contexto nigricanti subtem epidermidem hyalinam. Asci numerosi longe cylindrici, 140–160 × 11–14 μ, pariete crasso; basi unguiculiformes. Sporae 23–26 × 7.5–9.5 μ, imbricatae, uniseriatae, fusoideae, lutei-brunneae, 3-septatae, plerumque rectae et symmetricae, interdum inaequilaterales vel asymmetricae, acute angustatae, ad septa constricta, cellulis centralibus una vel duabus verticaliter septatae.

Specimen typicum in foliis *Agavis,* in Herbario Horti Botanici Noveboracensis, No. 21.

Perithecia 300–500 μ in diameter, appearing upon the surface as thickly scattered, black, circular spots, immersed in the cortex and erumpent through the cuticle as short cylindric ostioles; wall some 50 μ thick, composed of coarse black-walled parenchyma, commonly splitting into two layers. There is also a ring-like, clypeate, stromatic blackening of the tissues just beneath the cuticle (which remains hyaline) about 100 μ in thickness, which appears on the surface as a circular blackened spot.

Asci numerous, long-cylindric, with a thickened wall and a claw-like base, 140–160 × 11–14 μ, imbedded in numerous interthecial strips.

Spores overlapping uniseriate, fusoid, yellow-brown, 3-septate, mostly straight and symmetric or slightly inequilateral or asymmetric, acutely tapered toward the ends, slightly constricted at the central septum, usually one or sometimes two central cells with a vertical septum, 23–26 × 7.5–9.5 μ.

Collection: *21,* on *Agave (Type).*

This collection, in the New York Botanical Garden, bears no data, but is placed under *Pleospora Thuemeniana* Sacc. and is accompanied by a description. The spores have the same form as those of *P. pellita,* but are larger. The perithecia, however, are large, thick-walled and form a definite clypeus. This clypeus formation may be a reaction of the host, for other collections of *Pleospora* on *Yucca* and *Agave* show the same sort of clypeus but the perithecia contain quite different spores. The asci are also long and cylindric, with uniseriate spores and prominent interthecial strips, as found in species on woody substrata.

Portions of the type collection (438) of *P. Thuemeniana* Sacc., collected by Ravenel, on *Yucca aloifolia,* in 1876, have been ex-

amined in Thuem. Myc. Univ. 1846 (Riksmuseet), Roum. Fung. sel exs. 4959 (N.Y.B.G.) and the Sydow Herbarium (Riksmuseet). These all contain various Fungi Imperfecti but no *Pleospora* except for a few loose spores on the surface which might have belonged to *P. vulgaris*. The description gives the spores as three-septate and 18–20 × 7–8 μ.

Another collection in the N.Y.B.G. (*270*) on *Agave* shows identical perithecial development as in No. *21* but spores of the *P. media* type. Berlese, again, describes and figures spores (1, vol. 2: 6, *pl.* 6, *fig.* 2) which he says were taken from Saccardo's original material. The figures show more ellipsoid spores with more rounded ends than those found in No. *21* and they are colored greenish, but the dependability of this color is questionable. These spores are 3-septate and 18–21 × 7–9 μ. He shows no clypeus about the perithecia, and gives them as 250 μ in diameter. No. *21* may be the same as *P. Thuemeniana*, but in the absence of any definite evidence from authentic material it seems better to describe it as new. Because of the clypeus this species might be placed in *Phaeopeltosphaeria* Berl. but these clypeate blackenings occur in fungi of many different types and the type of this genus (*P. caudata*) does not seem to be related to *Pleospora clypeata*.

The origin of secondary septa, of the vulgaris type, in the central cells of such spores as those of *P. oligostachyae* and *P. diaporthoides* gives rise to 5-septate spores of the type found in the vulgaris or herbarum series, in contrast to those of *P. vagans* in the leptosphaeroid group. This type of septation has no doubt arisen at various times in various strains and is found in varying percentages in the spores of different collections.

PLEOSPORA BOLDOAE AND P. LACTUCICOLA

Collections whose spores show the transition from the 3- to the 5-septate condition are also difficult to distinguish from immature collections of species (*i.e., P. vulgaris*) whose spores are definitely 5-septate at maturity, for such immature spores show an irregular and progressive insertion of additional septa.

The following two species, therefore, are more or less arbitrary groupings of such transitional spore types.

PLEOSPORA BOLDOAE Speg., Fung. Chil. 87. 1910.

Illustration. Figure 12.

Perithecia 200–400 × 150–200 μ, somewhat flattened, formed beneath the periderm.

Asci cylindric-clavate, 70 × 12.5 μ, with a somewhat tapered base, and slightly thickened walls.

Spores biseriate, light yellow-brown, ellipsoid, 3-, sometimes 4- or 5-septate, straight or slightly inequilateral, mostly symmetric, ends bluntly tapered or broadly rounded, constricted at the central septum, sometimes at the secondary ones, either the central or end cells with vertical septa and often with secondary vulgaris-type transverse septa in one or, rarely, both central cells, 14–17 × 5.5–7 μ.

Collection: *22 (Type)*, on *Boldoa fragrans,* Chile.

The type collection of this species contains only a few perithecia in very poor condition. The spores are mostly 3-septate but may have an additional septum in either central cell and vertical septa in either the central or end cells. They differ from those of the following species in the light yellow-brown color.

PLEOSPORA LACTUCICOLA E. & E., Journ. Myc. 4: 64. 1888.

Illustration. Figure 13.

Perithecia globose or somewhat flattened, 200–300 μ in diameter, thickly scattered, immersed, erumpent as small papillate ostioles; wall 10–20 μ thick, composed of small-celled, dark, thick-walled parenchyma.

Asci clavate, apical wall slightly thickened, base claw-like, 75–110 × 10–15 μ, with rather numerous filiform interthecial strips.

Spores biseriate, ellipsoid to fusoid-ellipsoid, reddish-brown, 3- to 5-septate, straight or inequilateral to slightly curved, mostly symmetric, ends bluntly tapered or rounded, constricted at the central septum, one or both central cells often with vulgaris-like secondary septa, vertical septa sometimes present in end cells, 15–20 × 5.5–7(8) μ.

Collections: *52 (Type)* and *16,* on *Lactuca* and *Artemisia,* from New Jersey.

Ellis says that his *P. lactucicola* differs from *P. Bardanae* Niessl in smaller perithecia (175–200 μ) and smaller darker spores. The spores are dark and red-brown, but the perithecia are up to

300 μ and the spores to 20 μ. Ellis does not mention the additional septa in the central cells, but they are shown by Berlese (1, vol. 2, *pl.* 4, *fig.* 2). Collection *16* was placed in *P. diaporthoides* by Ellis, from which species it differs only in the insertion of the additional septa in the central cells.

<div align="center">

PLEOSPORA ATROMACULANS, P. SHEPHERDIAE AND

P. AMELANCHIERIS

</div>

There are several species with 3-septate spores which show certain characters typical of the wood-inhabiting forms and should probably be considered as the beginning of these tendencies. *P. clypeata,* already described, on *Agave,* has perithecia which are large and thick-walled and the asci are long-cylindric and imbedded in prominent interthecial strips. In *Pleospora atromaculans* (FIG. 15) and *P. findens* (FIG. 7) the spores are oblong in form, intermediate between the leptosphaeroid and vulgaris type, but do not seem to be related to *P. trichostoma.* In *P. atromaculans* the spores are red-brown and so suggest the vulgaris series, *P. findens,* on the other hand, has pale yellow-brown spores which are 3-septate and differ from *P. vagans* only in their cylindric form. *P. Shepherdiae* (FIG. 16) and *P. Amelanchieris* (FIG. 17) have spores which are characteristic in form of the vulgaris series and grade off into that series by the insertion of secondary septa.

PLEOSPORA ATROMACULANS Rehm, Ann. Myc. 6: 177. 1904.

Illustration.   Figure 15.

Perithecia 300–400(500) μ in diameter, thickly scattered, imbedded in the cortex, erumpent through the periderm, causing blackened angular ruptures 250–500 μ in diameter; walls rather thick (30–50 μ), of flattened brown parenchyma.

Asci numerous, clavate to cylindric-clavate, walls only slightly thickened, base claw-like, 70–80 (100 μ in desc.) × 9–11 μ, imbedded in a mass of interthecial strips.

Spores biseriate to obliquely uniseriate, oblong-ellipsoid or almost cylindric, red-brown, 3-septate (rarely 5-septate), ends bluntly rounded, constriction none or slight at central septum, one vertical septum in one of the central cells of less than 50 per cent of the spores, 14–17 × 5–5.5 μ.

Collection: *18,* on *Cornus,* N. Carolina (*Type*).

This species shows the characteristics of the forms on woody substrata, but *to* only a slight degree. The perithecia are rather large and thick-walled and cause rather wide ruptures of the periderm. The asci are numerous, somewhat cylindric and with abundant interthecial strips, but these characters are not as outstanding as in other woody forms. The spores have a characteristic almost cylindric form, with straight side walls and rounded ends, in which they differ from those of *P. diaporthoides.* These spores are occasionally 4-septate, in which case the additional septum is laid down in the end cell.

PLEOSPORA SHEPHERDIAE Pk., Rep. N. Y. St. Mus. **40**: 71. 1887.

Pleospora carpinicola E. & E. Proc. Acad. Nat. Sci. Phila. **1893**: 135.

Karstenula carpinicola (E. & E.) Berl. Ic. Fung. **2**: 4.   1900.

Pleospora juglandis E. & E. Bull. Torr. Bot. Cl. **24**: 279.   1897.

Illustration.   Figure 16.

Perithecia 250–700 μ in diameter, scattered or sometimes in small groups or confluent, globose or slightly flattened, sunken in the cortex, with small papillate ostioles erumpent through linear, angular or circular ruptures of the periderm; walls thick (30–100 μ), consisting of an outer layer of dark brown and an inner layer of hyaline parenchyma.

Asci numerous, long-cylindric, apical wall somewhat thickened, base claw-like, 85–180 × 7–11 μ, with numerous interthecial strips.

Spores uniseriate to overlapping uniseriate, oblong-ellipsoid or sometimes clavate, yellow-brown to light red-brown, 3-septate, ends broadly rounded, straight, symmetric or sometimes somewhat tapered below, one or two of the central cells with vertical septa in about half of the spores, rarely with a vertical septum in an end cell, 17.5–23 × 6–11 μ.

Collections: *164* (*Type*), *19, 20,* on *Shepherdia, Carpinus,* and *Juglans* from Kansas, New York and Ontario.

The three species united here are all very similar; they might be considered as varieties, but until a greater number of collections

demonstrates the constancy of the minor variations it is considered more practical to unite them.   They all show the large, thick-walled perithecia, with many cylindric asci imbedded in an abundance of interthecial stromatic tissue and characteristic of these woody forms.

The type (*164*) of *P. Shepherdiae* shows certain differences in the larger more flattened and more widely erumpent perithecia and the greater variability of the spores (FIG. 16a), which are often asymmetric, tapered below, or may occasionally show secondary septa, becoming 4–5-septate.

The type of *P. carpinicola* (*19*) shows smaller perithecia (250–500 μ) and rather small, narrow, regular spores (FIG. 16b), 18–21.5 × 6–7.5 μ.   The perithecia are sometimes confluent, but never grouped in stromata, not tomentose, and not red or white as suggested by Berlese in transferring this species to *Karstenula*.

The type of *P. Juglandis* (*20*) has the smaller perithecia (300–400μ) and regular spores (FIG. 16c) of *P. carpinicola,* but the spores [19–23 × 7.5–9(11) μ] are larger and the asci (160–180 × 9–11 μ) longer.

The following species commonly shows secondary septa and therefore is of the 5-septate type.   The spores show the transition to the vulgaris type of septation, bearing the same relationship to *P. Shepherdiae* that *P. lactucicola* bears to *P. diaporthoides.*

PLEOSPORA AMELANCHIERIS Wehm., Lloydia **9**: 206.   1946.

Teichospora praestipa Clem. ined. in Crypt. Form. Colo. 456.

Illustration.   Figure 17.

Inasmuch as a description has been given of this species in a previous paper (**9**) it will not be repeated here.   It differs from the preceding species in the large rimosely cracked and widely erumpent perithecia and in the larger spores (22–26 × 9–10.5 μ) which commonly show secondary septa of the vulgaris type in the central cells and sometimes vertical septa in the end cells. They are also more commonly constricted at the secondary septa.

Collection *165* (*Type*) and *166,* on *Amelanchier,* from Colorado and Wyoming.

COLLECTIONS CITED

1. *Pyrenophora hyphasmatis* E. & E., on rejected piece of rag, Pointe à la Hache, La., July 25, 1888, leg. A. B. Langlois (Fl. Ludoviciana No. 1434) (N.Y.B.G.) (*Type*). (Ellis cites the type as from St. Martinsville, La., July 1888, Langlois No. 1433, but this is no doubt the same coll.)

2. *Pyrenophora delicatula* Vestergr., on *Cerastium tomentosum*, Hort. Upsal., Suecica, May and June, 1896, Tycho Vestergren. 2 pkts. (*Cotypes*) (Riksmuseet).

3. *Pleospora calvescens* (Fr.) Tul., Syd. Myc. March. 661, on *Chenopodium album*, Berlin, July, 1884, P. Sydow (Riksmuseet).

4. *Pleospora calvescens* (Fr.) Tul., Rehm Asc. 439b, on *Atriplex nitens*, Elb-Ufer, bei Königstein (Sachsen), Aug., 1882, Kreiger (Riksmuseet).

5. *Pleospora calvescens* (Fr.) Tul., All. & Schnabl. Fung. Bav. 339, on *Chenopodium album*, München: Sendling, April, 1894, Schnabl. (Riksmuseet).

6. *Pleospora diaporthoides* E. & E., on *Bardana*, N. J. (Riksmuseet; Rehm Herb.). (There is also a label, *P. lactucicola* on *Coleosanthus californicus*; Pl. S. Cal. No. 5384, on this packet.)

7. *Pyrenophora pellita* (Fr.) Sacc., on *Papaver somniferum*, Fischerhaus bei Grossbehaltre, Kr. Westhavelland (?),* April 5, 1904, W. Kirschstein (Riksmuseet).

8. *Pleospora hysterioides* E. & E., on *Sporobolus asper*, Rockport, Kans., Feb. 15, 1893, Bartholomew No. 932 (N.Y.B.G.; Ellis coll.).

9. *Sphaeria pellita* Fr., on pavot somnifere, à la compagne de Mr Goethals—Delevigne près Courtrai (Riksmuseet, ex Herb. Syd., ex West. Herb. Cr. Belg. 652).

10. *Pleospora calvescens* (Fr.) Tul. f. *papaveracea* (DN.) Sacc., Rehm Asc. 736b, on *Papaver somniferum*, Mullrose (Brandenburg), 1909, P. Sydow (Riksmuseet).

11. *Pleospora pellita* (Fr.) Rab., Thuem. Herb. Myc. 356, on *Papaver somniferum*, Baden, bei Rastadt, Oct., 1875, leg. Schroeter (Riksmuseet).

12. *Pleospora pellita* (Fr.) Rab., Herb. Myc., II, 749, on herbaceous stem, Dresden, spring (Riksmuseet).

13. *Pleospora papaveracea* (de Not.) Sacc., Rehm Asc. 736, on *Papaver somniferum*, Ungarisch-Altenburg (Ungarn), April, 1883, Prof. Linhart (Riksmuseet).

14. *Pleospora pellita* (Fr.) Rab., on *Papaver Rhoes*, Neuchatel, May 22, 1871, leg. Morthier (Riksmuseet; Herb. Syd.).

15. *Pleospora diaporthoides* E. & E., on *Pastinaca sativa*, Newfield, N. J., July, 1890 (N.Y.B.G.: Ellis coll.) (*Type*).

16. *Pleospora diaporthoides* E. & E., on *Artemisia vulgaris*, Newfield, N. J., Aug. 20, 1896 (N.Y.B.G.: Ellis coll.).

17. *Pleospora oligostachyae* E. & E., on *Bouteloa oligostachya*, Rooks Co., Kans., Oct. 5, 1896, E. Bartholomew (N.Y.B.G.: Ellis coll. 2325) (*Type*).

18. *Pleospora atromaculans* Rehm, on *Cornus,* Blue Mts., N. Carolina, 1903, leg. Atkinson (Riksmuseet) (*Type*).

19. *Pleospora carpinicola* E. & E., on *Carpinus (americana)*, London, Canada, April, 1892 (N.Y.B.G.: Ellis coll. No. 1738) (*Type*).

20. *Pleospora juglandis* E. & E., on *Juglans nigra,* Rooks Co., Kans., May 12, 1897, E. Bartholomew (N.Y.B.G.: Ellis Coll. No. 2405) (*Type*).

21. *Pleospora Thuemeniana* Sacc., on *Agave* (N.Y.B.G.: Ellis coll. No data, but description given).

22. *Pleospora Boldoae* Speg., on *Boldoa fragrans,* Talcahuana, Chile, Jan., 1909 (La Plata Mus. No. 2182) (*Type*).

23. *Pleospora parvula* Berl., on *Clematis* (Lovere im Treo-Min ??),* Italia, May, 1900, Dr. Rehm (Riksmuseet: Herb. Rehm).

24. *Pleospora minuta* Romell inedit., on *Aira caespitosa, Phleum pratense,* Nrk. par Kumla: Kumla, June 19, 1885, M. A. 23, leg. L. Romell (Riksmuseet Herb. Lars Romell No. 16114).

25. Undetermined, on *Phleum,* Gottland: Bro, July 18, 1913, T. Vestergren (Riksmuseet: Fung. Suec.).

26. Undetermined, on *Phleum pratense,* Gottland: Bro, July 17, 1913, leg. T. Vestergren (Riksmuseet: Fung. Suec.).

27. Undetermined, on *Poa pratense,* Gottland: Bro, July 17, 1913, leg. T. Vestergren (Riksmuseet: Fung. Suec.).

28. *P. tenuis* ined., on *Melica ciliata,* Gottland: Bro, July 19, 1920, leg. T. Vestergren (Riksmuseet: Fung. Suec.).

29. Undetermined, on *Melica ciliata,* Gottland: Brissund, June 22, 1920, leg. T. Vestergren (Riksmuseet: Fung. Suec.).

30. Undetermined, on *Festuca rubra,* Gottland: Bro, June 17, 1920, leg. T. Vestergren (Riksmuseet: Fung. Suec.).

31. *Pleospora findens* E. & E., on *Andropogon virginica,* Newfield, N. J., Oct. 28, 1896 (N.Y.B.G.) (*Type*).

32. *Pleospora fuegiana* Speg., on *Poa Forsteri,* Chair Is., Tierra del Fuego, May, 1882 (La Plata Mus. 2179) (*Type*).

34. *Pleospora Forsteri* Speg., on *Festuca magellanica,* Isle de la Estados, March, 1882 (La Plata Mus. 2188) (*Type*).

35. *Pleospora mollis* Starb., on *Ephedra americana,* Jujuy, Argentina, Morene, Dec. 16, 1901, R. E. Fries (Riksmuseet: Exp. Suec. Chaco-Andinis No. 230) (*Type*).

52. *Pleospora lactucicola* E. & E., on *Lactuca,* Newfield, N. J., June 9, 1878, leg. Ellis (N.Y.B.G. 3061: Ellis coll. 297).

60. *Pyrenophora delicatula* Vestergr., on *Cerastium tomentosum,* Hort. Upsal. Suecica, April 14, 1899, leg. Vestergren (Riksmuseet: Vest. Micr. rar. sel. 107).

61. *Pleospora hysterioides* E. & E., on *Andropogon nutans,* Feb. 10, 1893, Kansas, leg. Bartholomew (N.Y.B.G.: Ellis coll. 915) (*Type*).

61a. Same data; second fungus.

120. *Pleospora Lecanora* (Fabre) Rehm, on *Salsola Tragus,* Kulm, N. Dakota, July 19, 1913, leg. J. Brenckle (Riksmuseet: Rehm Herb. ex Brenckle Herb. Fung. Dak. 240).

* Question marks indicate that the handwriting on the packet could not be deciphered and the translation given is extremely doubtful.

120a. Same data; second fungus.

164. *Pleospora Shepherdiae* Pk., on *Shepherdia canadensis,* Port Henry, N. Y., June 1866, leg. C. H. Peck (N. Y. St. Mus.) (*Type*).

165. *Pleospora Amelanchieris* Wehm., on *Amelanchier elliptica,* Hoback Canyon, Jackson, Wyo., July 16, 1940 (Wehm. Herb. 1141) (*Type*).

166. *Teichospora praestipa* Clem. inedit., on *Amelanchier oreophila,* Sulphur Springs, Colo., July 23, 1907 (Farl.: Clem. Crypt. Form. Colo. 456).

270. *Pleospora Thuemeniana* Sacc., on *Yucca* or *Agave,* Matamoras, Mexico, June 1895, leg. Dr. Egeling (N.Y.B.G.: Ellis coll.).

324. Undetermined, on *Calamagrostis,* Ad. Åre Jemtlandiae, June 27, 1930, leg. A. G. Eliasson (Riksmuseet: Fl. Suec.).

325. Undetermined, on *Elymus arenaria,* Suecia, litore inter Båstad et Malen, July 27, 1927, leg. A. G. Eliasson (Riksmuseet: Fl. Suec.).

329. Undetermined, on *Calamagrostis lanceolata,* Sueciae: prope viam inter Storlien et Storvallen Jemtlandiae, July 31, 1932, leg. A. G. Eliasson (Riksmuseet: Fl. Suec.).

332. Undetermined, on *Poa compressa,* Gottland: Bro, June 19, 1920, leg. T. Vestergren (Riksmuseet: Fung. Suec.).

335. *Pleospora tenuis* ined., on *Agrostis canina,* Gottland: Bro s;n, n,Bro, June 21, 1920, leg. T. Vestergren (Riksmuseet: Fung. Suec.).

339. Undetermined, on *Poa compressa,* Gottland: Bro, June 16, 1920, leg. T. Vestergren (Riksmuseet: Pl. Suec.).

352. *Pleospora papaveracea* (de Not.) Sacc., on *Papaver somniferum,* Syd. Myc. Germ. 791, Brandenburg bei Dammendorf bei Millrose, July 14, 1909, leg. P. Sydow (Riksmuseet).

421. *Pleospora coronata* Niessl, on *Carduus sphaerocephalus,* Italy: Conegliano, Aug., 1878 (La Plata Mus. 7185).

423. *Pleospora cereicola* Speg., on *Cereus quisco,* Batuco, Chile, Jan., 1909 (La Plata Mus. 2181) (*Type*).

424. *Pleospora Bardanae* Niessl, on *Althea rosae,* Italia: Conegliano, March, 1887 (La Plata Mus. 7183).

429. *Leptosphaeria aerea* Speg., on *Tillandsia bicolor,* Ensanada, La Plata, Argentina, Feb., 1881 (La Plata Mus. 2397) (*Type*).

438. *Pleospora Thuemeniana* Sacc., on *Yucca aloifolia,* Aiken, S. Carolina, Aest. 1876, leg. H. W. Ravenel (Riksmuseet: Thuem. Myc. Univ. 1846) (Farl.: Roum. Fung. sel. exs. 4850).

441. *Leptosphaeria obtusispora* Speg., on *Yucca gloriosa,* Flores, Buenos Aires, Argentina, March, 1881 (La Plata Mus. 2393) (*Type*).

447. Undetermined, on *Zizyphus jujuba,* Rohtak, India, May 18, 1945, leg. S. Ahmad (1218) (Wehm. Herb.).

449. *Pleospora relicina* (Fck.) on *Secale cereale,* Mahr-Weisskirchen, in ein Stoppelfelde, Feb. 29, 1912, leg. F. Petrak (Riksmuseet: Herb. Rehm).

450. *Pleospora relicina* Wint., on *Poa pratensis,* Mahr-Weisskirchen, Feb. 28, 1912, leg. F. Petrak (Riksmuseet: Herb. Rehm).

451. *Pleospora culmorum* (Cke.) Sacc., on *Triticum repens,* Stockholm: Djurgården May 8, 1914, leg. T. Vestergren (Riksmuseet: Micr. rar. sel.).

492. *Pleospora (diaporthoides),* on *Sueda fruticosa,* Ladhar, Shaikhupura, India, Aug. 16, 1945, leg. S. Ahmad (1415) (Wehm. Herb.).

EXPLANATION OF FIGURES

Representative spores of each respective collection were chosen and drawn by camera lucida, to scale, as indicated, in order to show variation and allow comparison.

FIG. 1. Spores of *Pleospora trichostoma* (Fr.) Ces. & de Not., from collection *450*. FIG. 2. Spores of *Pleospora mollis* Starb., from collection *35*. FIG. 3. Spores of *Pleosphaeria hyphasmatis* (E. & E.) Berl., from collection *1*. FIG. 4. Spores of *Pyrenophora delicatula* Vestergr., from collections *2* (*a*) and *60* (*b*). FIG. 5. Spores of *Pleospora calvescens* (Fr.) Tul., from collections *4* (*a*) and *5* (*b*). FIG. 6. *Pleospora pellita* (Fr.) Rab., from collections *10* (*a*), *424* (*b*), *14* (*c*), *120a* (*d*). FIG. 7. Spores of *Pleospora findens* E. & E., from collection *31*. FIG. 8. Spores of *Pleospora vagans* Niessl, from collections *24* (*a*), *25* (*b*), *23* (*c*), *32* (*d*), *34* (*e*), and *324* (*f*). FIG. 9. Spores of *Pleospora oligostachyae* E. & E., from collections *8* (*a*), *61a* (*b*), and *17* (*c*). FIG. 10. Spores of *Pleospora diaporthoides* E. & E., from collections *6* (*a*), *15* (*b*), *429* (*c*), and *423* (*d*). FIG. 11. Spores of *Pleospora diaporthoides,* from collections *447* (*a*) and *492* (*b*). FIG. 12. Spores of *Pleospora Boldoae* Speg., from collection *22*. FIG. 13. Spores of *Pleospora lactucicola* E. & E., from collections *16* (*a*) and *52* (*b*). FIG. 14. Spores of *Pleospora clypeata,* from collection *21*. FIG. 15. Spores of *Pleospora atromaculans* Rehm, from collection *18*. FIG. 16. Spores of *Pleospora Shepherdiae* Pk., from collections *164* (*a*), *19* (*b*), and *20* (*c*). FIG. 17. Spores of *Pleospora Amelanchieris* Wehm., from collection *166*.

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NOTES AND BRIEF ARTICLES

SOME COPROPHILOUS ASCOMYCETES FROM PANAMA [1]

SAMUEL L. MEYER AND VESTA GREEN MEYER

In 1944–45, the senior author was on duty at the Army School of Malariology in the Panama Canal Zone for about sixteen months. Field work in connection with the teaching and research activities of the School provided an opportunity to collect dung at various localities in the Canal Zone and the Republic of Panama.

The dung was collected in the field, dried, packeted, and shipped to The University of Tennessee. Permission for such material to enter the United States was granted by officials of the Bureau of Entomology and Plant Quarantine of the United States Department of Agriculture.

When the senior author returned to The University of Tennessee in 1946, a taxonomic investigation of the coprophilous Ascomycetes of Panama was begun. The species reported here constitute the first published contribution in that study.

The dried samples of dung were moistened with distilled water and placed on moist paper toweling in glass culture dishes of various sizes and depths, depending upon the size of the sample. Water was added as needed to keep the dung moist. Examination of each sample for coprophilous Ascomycetes was made at intervals over a period of four to five months when the substrata were burned. Dried specimens of all species included in the list have been placed in the herbarium of The University of Tennessee.

For the convenience of those interested, this report follows a taxonomic arrangement. The distribution data for some species may be incomplete; however, the general distribution pattern for each species is fairly accurately defined. Nomenclature of the Discomycetes is according to Seaver (**1942**); that for the Pyreno·mycetes is according to Cain (**1934**).

The species listed may be grouped into some interesting categories:

1. Species new to Panama: So far as the writer is aware, none of the species listed here has been reported previously from the Panama Canal Zone or the Republic of Panama.

2. Species of widespread occurrence in the United States but apparently new to the Caribbean region: *Chaetomium bostrychodes* Zopf, *Sordaria decipiens* Winter, *S. vestita* Zopf, *Pleurage collapsa* Griff., *Sporormia minima* Auersw., *S. herculea* Ell. & Ev.

3. Species reported with a northern distribution in the United States but new to the Caribbean region: *Chaetomium spirochaete* Palliser.

4. Species reported from the United States and Bermuda but new to the Caribbean region: *Ascobolus immersus* Pers.

5. Species involved in a noteworthy extension of geographical range: *Ascodesmis microscopica* Seaver, *Sordaria appendiculata* Auersw., *Delitschia Marchalii* Berl. & Vogl.

6. Species reported on substrates other than those previously recorded: *Ascodesmis porcina* Seaver, *Chaetomium bostrychodes* Zopf, *C. spirochaete* Palliser.

DISCOMYCETES

Ascodesmis microscopica Seaver. No. 17908, on horse dung, Salud, Republic of Panama, April 5, 1945. Seaver (**1928**: 80) reports the distribution: New York; also in Europe.

Ascodesmis porcina Seaver. No. 17907, on horse dung, La Mesa, Republic of Panama, April 1, 1945; 17909, on horse dung, El Valle, Republic of Panama, April 1, 1945. Seaver (**1928**: 80) records the substrate and distribution: grown in the laboratories of The New York Botanical Garden on pig dung from Porto Rico. Horse dung appears to be a new substrate for this species.

Ascobolus immersus Pers. No. 17904, on cow dung, Chilibre, Republic of Panama, March 28, 1945; 17905, on horse dung, El Valle, Republic of Panama, April 1, 1945; 17906, on horse dung, Salud, Republic of Panama, April 5, 1945. Seaver (**1928**: 83) gives the distribution: New York to Colorado and Bermuda, probably throughout North America; also in Europe. This spe-

cies has also been collected in Quebec (Mains, Overholts, and
Pomerleau, **1939**: 730), Virginia (Betts and Meyer, **1940**; Meyer,
1943: 327; Wilson, **1947**: 375), and Tennessee (Meyer, **1941**:
402).

Saccobolus Kerverni (Crouan) Boud.   No. 17844, on horse
dung, Limon, Canal Zone, March 5, 1945; 17845 and 17847, on
cow dung, 17846, on horse dung, Juan Mina, Canal Zone, March
11, 1945; 17848, on horse dung, Buenos Aires, Republic of
Panama, March 22, 1945; 17849, on horse dung, and 17850, on
goat dung, Santa Rosa, Republic of Panama, March 24, 1945;
17851, on horse dung, and 17852, on cow dung, Chilibre, Republic
of Panama, March 28, 1945; 17853, on horse dung, La Mesa,
Republic of Panama, April 1, 1945; 17854, on horse dung, El
Valle, Republic of Panama, April 1, 1945; 17855, on cow dung,
Las Uvas, Republic of Panama, April 1, 1945; 17856 and 17857,
on cow dung, Lagarto, Republic of Panama, April 5, 1945; 17860,
on cow dung, Lagarto, April 6, 1945; 17858, on cow dung, and
17859, on horse dung, Salud, Republic of Panama, April 5, 1945.
Seaver (**1928**: 93) records the range of this species from New
York to Colorado, Bermuda, and Porto Rico, probably common
throughout North America; also in Europe.   Seaver (**1942**: 308)
notes a range extension to Quebec.   This species has also been
found in Florida (West, **1941**: 40), Tennessee (Meyer, **1941**:
403), and Virginia (Betts and Meyer, **1940**; Meyer, **1943**: 327;
Wilson, **1947**: 375).

Ascophanus argenteus (Curr.) Boud.   No. 17910, on cow dung,
Lagarto, Republic of Panama, April 5, 1945.   Seaver (**1928**: 115)
lists the distribution: New York to Colorado and Porto Rico;
also in Europe.   The range has been extended to Michigan
(Seaver, **1942**: 310) and Virginia (Wilson, **1947**: 375).

Ascophanus carneus (Pers.) Boud.   No. 17911, on cow dung,
Chorrera, Republic of Panama, March 6, 1945; 17912, on cow
dung, Port of Chorrera, Republic of Panama, March 13, 1945;
17913, on horse dung, Juan Mina, Canal Zone, March 11, 1945;
17914, on cow dung, Las Uvas, Republic of Panama, April 1,
1945.   Seaver (**1928**: 116) gives the distribution: New York to
North Dakota, Florida, Colorado, Bermuda, and Porto Rico;
also in Europe.   A collection was made on the Mycological Society

Foray in Quebec (Mains, Overholts, and Pomerleau, **1939**: 730) and the species has been found in Tennessee (Meyer, **1941**: 403). Seaver (**1942**: 310) reports the range to Colombia, Venezuela, and Ontario.

Ascophanus granulatus (Bull.) Speg. No. 17875, on horse dung, El Valle, Republic of Panama, April 1, 1945. Determination by F. J. Seaver. Seaver (**1928**: 117) records the distribution: Connecticut to Iowa, South Carolina, and Mexico; also in Europe. The known range has been extended to include Winnepeg, California, Porto Rico, Dominican Republic, Venezuela, and Bermuda (Seaver, **1942**: 310).

PYRENOMYCETES

Sordaria fimicola (Rob.) Ces. & De Not. No. 17821, on horse dung, Chorrera, Republic of Panama, March 6, 1945; 17822, on horse dung, Chorrera, Republic of Panama, March 12, 1945; 17823, on horse dung, Port of Chorrera, Republic of Panama, March 13, 1945; 17824, on horse dung, Buenos Aires, Republic of Panama, March 22, 1945; 17825, on horse dung, Chilibre, Republic of Panama, March 28, 1945; 17826, on horse dung, Las Uvas, Republic of Panama, April 1, 1945; 17882, on horse dung, El Valle, Republic of Panama, April 1, 1945. Griffiths and Seaver (**1910**: 67) record this species from Vermont to Oregon, Arizona, and Alabama; also in Europe. They place it in the genus *Fimetaria* Griffiths and Seaver. It has been collected since in St. Croix, Virgin Islands (Seaver, **1925**: 5), Michigan (Ames, **1930**: 319), Ontario (Cain, **1934**: 18), and Virginia (Betts and Meyer, **1940**; Meyer, **1943**: 330; Wilson, **1947**: 376).

Sordaria appendiculata Auersw. No. 17887, on horse dung, Chorrera, Republic of Panama, March 12, 1945; 17888, on horse dung, Chorrera, Republic of Panama, March 6, 1945; 17889, on horse dung, Las Uvas, Republic of Panama, April 1, 1945. This species is listed as *Pleurage superior* D. Griff. by Griffiths and Seaver (**1910**: 74) and reported from Montana. It has also been found in Ontario (Cain, **1934**: 35).

Sordaria decipiens Winter. No. 17890, on horse dung, Las Uvas, Republic of Panama, April 1, 1945; 17891, on horse dung, Chorrera, Republic of Panama, March 12, 1945. Listed as

Pleurage decipiens (Wint.) Kuntze by Griffiths and Seaver (**1910**: 77) who give the range: Vermont to Montana, Arizona, and Alabama; also in Europe. It has been collected in Michigan (Ames, **1930**: 319), Ontario (Cain, **1934**: 44), and Virginia (Meyer, **1943**: 331; Wilson, **1947**: 376).

Sordaria vestita Zopf. No. 17836, on cow dung, Port of Chorrera, Republic of Panama, March 13, 1945; 17837, on horse dung, Santa Rosa, Republic of Panama, March 24, 1945; 17838, on horse dung, La Mesa, Republic of Panama, April 1, 1945; 17839, 17840, and 17841, on cow dung, Salud, Republic of Panama, April 5, 1945; 17843, on horse dung, Salud, Republic of Panama, April 6, 1945. The distribution is given as New York to Oregon, Arizona, and Louisiana and also in Europe by Griffiths and Seaver (**1910**: 76) who put the species in the genus *Pleurage* Fries. It is listed among the fungi collected on the Foray in Quebec (Mains, Overholts, and Pomerleau, **1939**: 731). This species has been found in Tennessee (Meyer, **1941**: 404), Ontario (Cain, **1934**: 45), and Virginia (Meyer, **1943**: 331; Wilson, **1947**: 376).

Pleurage collapsa Griff. No. 17835, on horse dung, El Valle, Republic of Panama, April 1, 1945. Griffiths and Seaver (**1910**: 80) place this species in the genus *Pleurage* Fries and record the distribution: New York to Alabama. The species has also been found in Saskatchewan (Cain, **1934**: 59) and Virginia (Wilson, **1947**: 376).

Bombardia arachnoidea (Niessl) Cain. No. 17830, on cow dung, and 17831, on horse dung, Juan Mina, Canal Zone, March 11, 1945; 17832 and 17833, on cow dung, Las Uvas, Republic of Panama, April 1, 1945; 17834 and 17883, on cow dung, Lagarto, Republic of Panama, April 5, 1945; 17884 and 17885, on cow dung, Chilibre, Republic of Panama, March 28, 1945. Griffiths and Seaver (**1910**: 75) place this species in the genus *Pleurage* Fries and report the distribution: New York to New Jersey; also in Europe. It has been found in Porto Rico (Chardon, **1921**: 294–295), St. Thomas (Seaver, **1924**: 6), Ontario (Cain, **1934**: 73), Tennessee (Meyer, **1941**: 404), and Virginia (Meyer, **1943**: 330; Wilson, **1947**: 376).

Delitschia Marchalii Berl. & Vogl. No. 17879, on horse dung, Port of Chorrera, Republic of Panama, March 13, 1945; 17880,

on horse dung, La Mesa, Republic of Panama, April 1, 1945; 17881, on horse dung, Salud, Republic of Panama, April 5, 1945. Griffiths and Seaver (**1910**: 82) record the distribution: New Jersey; also in Europe. Griffiths and Seaver (**1910**: 82) list as the substrate: rabbit dung. Cain (**1934**: 80) records the species from Ontario with the observation that it is fairly common on the dung of various animals but especially rabbit.

Sporormia minima Auersw. No. 17865, on horse dung, Limon, Canal Zone, March 5, 1945; 17866, on horse dung, Chorrera, Republic of Panama, March 6, 1945; 17867, on cow dung, Chorrera, Republic of Panama, March 6, 1945; 17868, on horse dung, Chorrera, Republic of Panama, March 12, 1945; 17869, on cow dung, Port of Chorrera, Republic of Panama, March 13, 1945; 17870 and 17871, on horse dung, Santa Rosa, Republic of Panama, March 24, 1945; 17872, on cow dung, Chilibre, Republic of Panama, March 28, 1945; 17873 and 17874, on cow dung, Salud, Republic of Panama, April 5, 1945. Reported distribution: Vermont to Oregon, Arizona, and Louisiana; also in Europe (Griffiths and Seaver, **1910**: 94). It was collected in Ontario (Cain, **1934**: 94), in Quebec on the Mycological Society Foray (Mains, Overholts, and Pomerleau, **1939**: 731), and in Virginia (Meyer, **1943**: 331; Wilson, **1947**: 377).

Sporormia herculea Ell. & Ev. No. 17864, on horse dung, Santa Rosa, Republic of Panama, March 24, 1945. Griffiths and Seaver (**1910**: 88) record the distribution: Rhode Island to Texas. It has been reported from Ontario (Cain, **1934**: 110) and Virginia (Wilson, **1947**: 377).

Chaetomium bostrychodes Zopf. No. 17815, on cow dung, Salud, Republic of Panama, April 6, 1945; 17816, on rabbit dung, and 17817, on horse dung, Chilibre, Republic of Panama, March 28, 1945; 17818, on horse dung, Port of Chorrera, Republic of Panama, March 13, 1945; 17819, on horse dung, Buenos Aires, Republic of Panama, March 22, 1945. Reported range: New York to Louisiana; also in Europe (Palliser, **1910**: 62). This species has also been found in Iowa on open pasture land, one to three inches below the soil surface (Paine, **1927**: 254). It has been collected in Tennessee (Meyer, **1941**: 403) and Virginia (Meyer, **1943**: 330; Wilson, **1947**: 375). Palliser (**1910**: 62)

lists the following substrata: dog dung, sheep dung, old shoe, potatoes, and decaying portions of animals. Meyer (**1943**: 330) and Wilson (**1947**: 375) found this species on deer mouse dung while Wilson (**1947**: 375) also collected it from horse dung. Apparently, it had not previously been found on cow and rabbit dung.

Chaetomium spirochaete Palliser. No. 17820, on goat dung, Santa Rosa, Republic of Panama, March 24, 1945. Reported range: New Jersey to Iowa (Palliser, **1910**: 61). This species has been found growing on moist decayed paper and cotton root in a moist chamber but apparently has not been previously reported from dung.

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WESTERN FUNGI—I

Wm. Bridge Cooke

(WITH 22 FIGURES)

A. Some miscellaneous species from Mount Shasta

1. Peyronelia sirodesmoides Cif. & Frag. In 1927 Ciferri and Fragoso proposed this genus and species for a black mold growing on rotting wood in the Dominican Republic. It was cespitose, black, woolly, had sterile creeping hyphae which were olivaceous, septate, and 3–4.5 μ in diameter. It did not have conidiophores, or these structures were indistinct, being merely the broadened base of the primary conidium. The conidia were black, 1–8 but mostly 2–5 in a chain, fusoid, 3–10-septate, 28–60 × 7–15 μ (averaging 35–45 × 9–12 μ), easily separating at a narrow isthmus 2–3 μ in diameter.

On Mount Shasta a fungus was found on dead sticks of *Holodiscus discolor* var. *glabrescens* Heller which fits this description well. The spores in the chains are exogenous, 16–43.5 × 10–14 μ, 1–5-septate, with the isthmus between the spores 5–6 μ in diameter. In some instances there seem to be rather distinct conidiophores.

The Mount Shasta collection was made on July 16, 1946, on a ridge north of Horse Camp at about 9000 feet elevation, W. B. Cooke *18253*. Despite the wide geographic separation of these two collections there does not seem to be enough difference on which to base a specific segregation.

2. Arthrinium bicorne Rostrup. Material of this species was found forming black sooty areas on dead culms of *Juncus balticus* var. *montanus* Engelm. at Horse Camp, Mt. Shasta, Siskiyou Co., Calif., June 24, 1946, W. B. Cooke *18032*. Mycobiota of North America *280*.

The spores are produced on erect conidiophores up to 200–300 μ long which are divided into a number of short cells from each of which one or two spores are produced on short, lateral processes. The spores are arranged like a row of shingles on the conidiophore. The spores are black, two-horned, smooth, one-celled. Their width from tip of horn to tip of horn is 20–40 μ; the dimensions of the spores exclusive of the horns are 8–13.5 $\times$ 13.4–16.7 μ; the horns are 7.5–13.4 $\times$ 2.5 μ. The specimen was identified by Lee Bonar.

3. Dendryphium pini von Hoehnel. A brown mold on Shasta Fir bark is assigned to this species. On the substratum the mold develops prostrate ropes of brown hyphae. These ropes are 50–75 μ in diam. and are composed of mostly granular incrusted hyphae 1.5–4 μ in diameter, although a few hyphae associated with them are smooth. The smaller hyphae are paler in color. These hyphae are branched and septate. The spores remain in chains until maturity. The chains are 2–20 spores long and are usually branched. The apparently erect conidiophores have roughened walls. The spores are dark brown at maturity, and have both thick and roughened walls. At first they are light brown. They are 1-septate, finally 2–3-septate and measure 10.5–18 $\times$ 5.5–7 μ.

According to the description published in Saccardo (Syll. Fung. 22: 1398), the spores are 16 $\times$ 5.5–6 μ, 2–4-septate, finally 4-septate. One wonders if "celled" were intended instead of "septate." *Dendryphium cladosporioides* Ell. & Ev. fits this material well except that it is reported from tomato vines and was collected in Louisiana. It is possible that these three collections may represent the same species.

The Mount Shasta material was obtained from bark of *Abies magnifica* var. *shastensis* Lemmon near Horse Camp, 8000 ft., June 1946, W. B. Cooke *18078*.

4. RAMULARIA MIMULI Ell. & Kell. Spots epiphyllous, becoming confluent, covering entire leaves and even shoots; spores hyaline, 0–3-septate, 18–(25)–33 × 3–4.5 μ.

On *Mimulus tilingii* Regel. In the overflow of a small spring above Panther Creek Meadows, 9000 feet, White Bark Pine Zone, Aug. 29, 1946, W. B. Cooke *18426;* in lower part of Panther
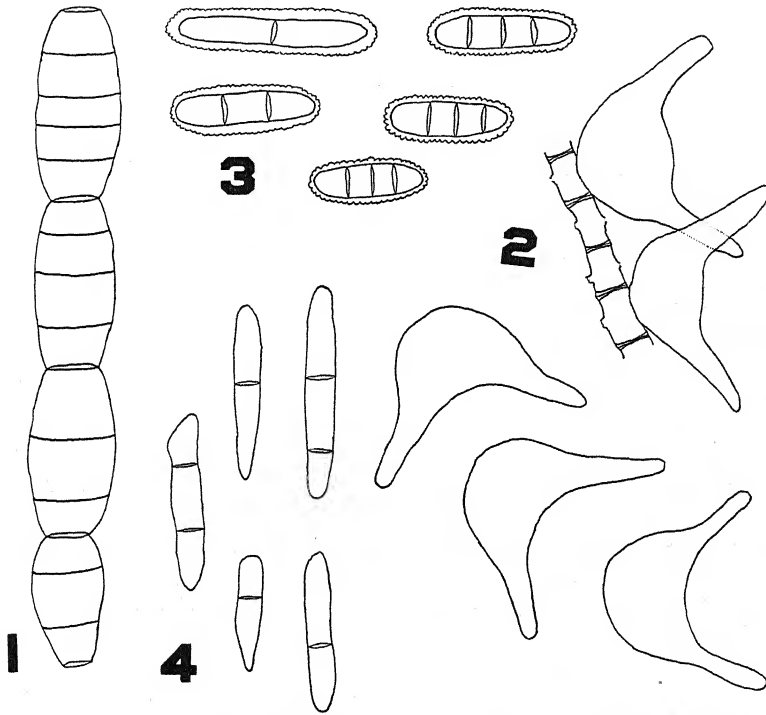
FIG. 1. Spores of *Peyronelia sirodesmoides.* FIG. 2. Spores of *Arthrinium bicorne.* FIG. 3. Spores of *Dendryphium pini.* FIG. 4. Spores of *Ramularia mimuli.*

Creek Meadows, 7000 ft., Shasta Fir Zone, Aug. 15, 1947, W. B. Cooke *20446.* Distributed in Mycobiota of North America No. *221.* Also observed in the large colony of this host around the springs above Horse Camp at 8250 ft. in the White Bark Pine Zone.

Only one species of *Ramularia* has been described on *Mimulus* in North America. It was first reported from Nebraska and the

spores from this collection were described as 30–40 × 3 μ. A later collection from Yellowstone National Park was reported to have larger spores, 40–96 × 4–5 μ. The above collections from Mt. Shasta have generally smaller spores than either of these specimens. It is felt that not enough material is yet available for more than mentioning the existence of this fungus complex.

5. **Ramularia claytoniae** W. B. Cooke, sp. nov. Maculis foliicolis, amphigenis, confluentibus; hyphis conidiophoris gregariis vel fasciculatis, 15–20 × 2 μ, hyalinis; conidiis catenulatis, hyalinis, 20–35 × 1.5–2.5 μ, 0–3-septatis.

Hab. in foliis vivis *Claytoniae sibericae,* Big Springs, prope McCloud, Siskiyou Co., Calif., Jul. 25, 1946. W. B. Cooke **18279.**

Spots on leaves becoming confluent, amphigenous; conidiophores in clusters or appearing singly through the epidermis or occasionally through stomata, up to 20 μ long by 2 μ wide, hyaline; conidia in chains, hyaline, 20–35 × 1.5–2.5 μ, 0–3-septate.

Type collected on *Claytonia siberica* L. in the overflow from the Big Springs of the McCloud River, NW 1/4, S. 14, T. 39 N., R. 2 W., Siskiyou Co., Calif., July 25, 1946, W. B. Cooke *18279.*

So far as either Lee Bonar or the writer can find there is no reference in the literature to a species of *Ramularia* occurring on any member of the Portulacaceae.

6. **Ramularia pentstemonis** W. B. Cooke, sp. nov. Maculis foliicolis, epiphyllis, non vel raro confluentibus; hyphis conidiophoris fasciculatis, 50–200 × 3 μ, hyalinis; conidiis plus minusve catenulatis, hyalinis, non septatis, 8–18 × 3–4.5 μ.

Hab. in foliis vivis *Pentstemonis shastensis,* 1850 m., Mt. Shasta, Siskiyou Co., Calif., Jul. 18, 1947. W. B. Cooke **20207.**

Spots white, scattered, surrounded by red-purple discolored areas; conidiophores hyaline, cespitose, emerging from stomata on upper surface of leaves, 50–300 × 3 μ, breaking up into conidia, or conidia produced terminally, or in chains; conidia hyaline, 8–18 × 3–4.5 μ, non-septate, rare in some spots, common in others. Conidiophores straight to flexuous, septate, some appearing geniculate, some occasionally branched.

Type collected in Wagon Camp Meadows, 5700 ft., Mt. Shasta, Sierra Mixed Conifer Zone, July 18, 1947, W. B. Cooke **20307.** On *Pentstemon shastensis* Keck. Occasionally plants were found to be heavily infected. Excessive humidity near the base of the plants may account for the poor conidial production and the rich

conidiophoral development with some branching to be found on more basal leaves.

No records of a species of *Ramularia* on *Pentstemon* have been found in the various host indices consulted.

7. Cylindrosporium smilacinae Ell. & Ev. and Cylindrosporium veratrinum Sacc. & Wint. On neighboring colonies of the host plants, *Smilacina stellata* (L.) Desf. and *Veratrum californicum* Durand, these two fungi caused heavy infection in the summer of 1947 at Wagon Camp, 5700 ft., Mt. Shasta. Morphologically these fungi appear distinct. Cross-inoculation studies are required to determine whether they represent only different reactions of the same fungus to different hosts.

C. smilacinae has spores $67–90.5 \times 2.5–3.5 \mu$, 1–2-septate, hyaline, more or less curved, produced in large loose masses.

C. veratrinum has spores $87.1–117.3 \times 3.5–4.5 \mu$, 2-septate, cylindric, falcate at one end in most cases, produced in small, compact, barrel-shaped masses which may become confluent for several mm.

The more irregular shape of the spores in *C. smilacinae* may be attributed to their method of production in large, irregular masses which on macroscopic examination of the leaf surface appear as whitish areas in the blackened infected areas of the leaf. The small, barrel-shaped masses of conidia in *C. veratrinum* appear as pink spots on blackened infected areas.

The original description of *C. veratrinum,* quoted in Saccardo, Sylloge Fungorum, describes the spores as being $70–90 \mu$ long. The material from Mt. Shasta noted above has longer spores, as does another collection observed recently. In July 1946, George Nyland obtained at Chinook Pass, Yakima Co., Washington, material assigned to this species by C. G. Shaw, on *Veratrum viride* Ait. (W.S.C. pl. p. *17446*). The spores of this specimen measure up to 170μ long although some spores fall within the range described by Saccardo and Winter.

This material again brings up the question of polymorphic species of leaf parasites of wild hosts. More extensive series of collections will be necessary to determine whether we are dealing here with only one species or with a group of closely related species.

8. Phyllosticta melanoplaca Thüm. Pycnidia assigned to this species by Lee Bonar were found associated with *Cylindro-*

sporium veratrinum on infected leaves of *Veratrum californicum* collected at Wagon Camp, Mt. Shasta. It has small, bacillar spores 2.5–4 × 1.0–1.5 μ. There is no indication that it is associated with the life cycle of the *Cylindrosporium*.

9. DOTHIORELLA MAGNIFRUCTA (Pk.) Petrak & Sydow (*Phoma magnifructa* Peck). Material referred by Lee Bonar to this species was collected on April 8, 1947 by W. B. and V. G. Cooke, W. B. Cooke *19336,* Mycobiota of North America *276,* on cones of *Libocedrus decurrens* Torr. lying on the ground along the Mud Creek Dam road a mile north of McCloud on the south side of Mt. Shasta.

This species forms small stromata in which 5–20 pycnidia are embedded. The pycnidiospores are cylindric, hyaline, non-septate, 18–22 × 3.2–3.8 μ

10. SELENOPHOMA LINICOLA T. C. Vanterpool. Pycnidia on the surface of stems the summer following the death of the above-ground portions of the host. Spores lunate, hyaline, non-septate, 16–22 × 2.9–3.5 μ.

On dead stems of *Iliamna bakeri* (Jeps.) Wiggins in the chaparral along the Shasta Snowline Highway, 4500 ft., Mt. Shasta, July 12, 1946, W. B. Cooke *18233.*

Morphologically this material is too close to *S. linicola* to establish it as a distinct species, although reference to collections of material published in the literature gives a precedent for such treatment. Cross-inoculation studies have not been made on it and it does not appear to be pathogenic since it was found on year-old litter.

11. SEPTORIA LUNELLIANA Sacc. Several plants of *Carex fracta* Mkze. at MacBride Springs Public Camp, 5000 ft., Mt. Shasta, were found to be heavily infected with this species on July 3, 1947, W. B. Cooke *20226.* The spots are white, surrounded by brown discolored areas which become confluent for large areas on the leaf. Pycnidia are scattered in the spots. The spores are 3-septate, hyaline, 55–77 × 3–3.5 μ. This specimen was identified by Roderick Sprague and distributed as no. *278* in Mycobiota of North America.

12. SEPTORIA AROMATICA Kabat & Bubak. Lesions at first yel-

low, then blackened with areas in which black pycnidia are located. These areas are not usually confluent and the pycnidia are scattered in them, but whole leaf segments may appear to become blackened. Pycnidia black, 75–100–$150\,\mu$ in diameter. Spores straight to curved, 1–3-septate, 63.5–80.4×3.5–$4\,\mu$, hyaline.

On *Ligusticum grayi* C. & R. along Panther Creek below the meadows, 7000 ft., Mt. Shasta, Aug. 15, 1947, W. B. Cooke *20441*.

No species of *Septoria* were found listed on *Ligusticum grayi* nor on related species from Western North America and a check was made on those species reported in Saccardo on umbelliferous hosts. From the sixty-four names found, seven species fall into or near the spore-size range noted above. The description of the species to which this collection is assigned fits this collection better than any of the other six.

13. **Phyllosticta crustosa** Lee Bonar & W. B. Cooke, sp. nov. Maculis hypophyllis, atris, confluentibus et totum folium saepe occupantibus; hyphis atris, in epidermide crustosis; pycnidiis globosis, sparsis vel gregariis, subepidermalibus, 90–$130\,\mu$ diam., pallide brunneis; ostiolo non papillato, poroideo; pycnidiosporulis bacillaribus, unicellularibus, hyalinis, 5.7×0.5–0.7–$1\,\mu$; conidiophoris simplicibus, hyalinis, 8–13 μ longis.

Hab. in foliis vivis *Kelloggiae galioidis,* 1850 m., Sisson Southern Trail, Mt. Shasta, Siskiyou Co., Calif., Aug. 22, 1947. W. B. Cooke **20471.**

Hypophyllous as blackish spots on leaves, distinct specks becoming confluent and often covering the entire leaf; blackening due to closely packed, sinuous dark hyphae in epidermal cells which form a black crust; pycnidia scattered, subepidermal, arising beneath the crust, light brown in color; ostiole poroid, non-papillate, conidia bacilliform, 1-celled, hyaline, 5–7×0.5–0.7–$1\,\mu$; conidiophores simple, hyaline, 8–$13\,\mu$ long.

Type collected on leaves of *Kelloggia galioides* Torr., W. B. Cooke **20471**, Sisson Southern Trail, Mt. Shasta, Siskiyou Co., Calif., Aug. 22, 1947. A second collection is filed at the Herbarium of the University of California: Rock Creek, 1.6 miles S.E. of Dean's Valley, S.E. of Meadow Valley, Plumas Co., Calif., Aug. 11, 1947. C. R. Quick *47–109*.

The fact that *Kelloggia* leaves were infected with some sort of disease was noted during the several years that the writer hiked Mount Shasta trails. However, after several futile attempts, fruiting material of the fungus was collected only in 1947.

Records of fungi on *Kelloggia* are not frequent and no record of a fungus such as this has been found in the literature. The writer is indebted to Lee Bonar for the above English description.

In 1889 Ellis and Everhart described *Hainesia borealis* from material of diseased *Galium boreale* L. collected at Kamloops, British Columbia, by J. Macoun. Material of this species was collected by R. Sprague, G. W. Fischer and J. P. Meiners on *Galium boreale* below Teton Pass in Teton Co., Wyoming near the Idaho line on Aug. 13, 1948. This material is housed in the Herbarium of the Dept. of Plant Pathology, State College of Washington as No. *17452*. The disease symptoms of this specimen look very much like those on young specimens of the Mount Shasta material but the spores are produced in acervuli rather than in pycnidia.

Such a situation was noted by C. L. Shear and B. O. Dodge in *Pezizella lythri* (Desm.) Shear & Dodge. In a paper entitled "The life history and identity of *'Patinella fragariae,'* *'Leptothyrium macrothecium,'* and *'Peziza oenotherae'* " (Mycologia **13** : 135–170, 1921), they recognized under *Pezizella lythri* two types of imperfect stages. The first of these was *Hainesia lythri* (Desm.) v. Höhn. It represented the conidial stage, and thirteen synonyms were listed for it. The second of these, the pycnidial stage, was *Sclerotiopsis concava* (Desm.) Shear & Dodge under which twelve earlier names were placed in synonymy. It is possible that *Hainesia borealis* Ell. & Ev., which has spores borne in acervuli and measuring 5–7 × 0.5–1 μ, and *Phyllosticta crustosa* Bonar & W. B. Cooke, which has spores borne in pycnidia and measuring 5–7 × 0.5–1 μ, are similarly two types of imperfect stage of an as yet unidentified or unknown ascomycetous fungus.

14. *Hysterium acuminatum* Fr. var. *alpinum* Rehm. Rehm referred material collected on *Pinus cembra* in the Tyrol to this variety in his Ascomycetes No. *125*. Three collections from Mount Shasta have been referred to this category by the writer and by Lee Bonar. They agree well with the description given in Saccardo (Syll. Fung. 2: 746. 1883). This species has been collected on bark of *Tsuga mertensiana* (Bong.) Sarg., W. B. Cooke *8663,* Aug. 24, 1937, at Panther Creek; on wood of *Abies magnifica* var. *shastensis* Lemmon, W. B. Cooke *10148,* June 23, 1938, at

Horse Camp; and on boards of *Pinus ponderosa* Dougl. at Horse Camp, 8000 ft., W. B. Cooke *18228, 18274,* July 1946.

The hysterothecia are 0.5–0.8 mm. long, dull black, superficial, filled with asci in all stages of development, $105–120 \times 7–8 \mu$; the spores are brown, smooth, uniseriate, constricted at the middle septum, $13–15 \times 6 \mu$. The spores are at first hyaline, develop a central septum before pigmentation starts, have most of their pigmentation before the second and third septa develop, and are finally dark-brown and 4-celled.

5

7　　**6**
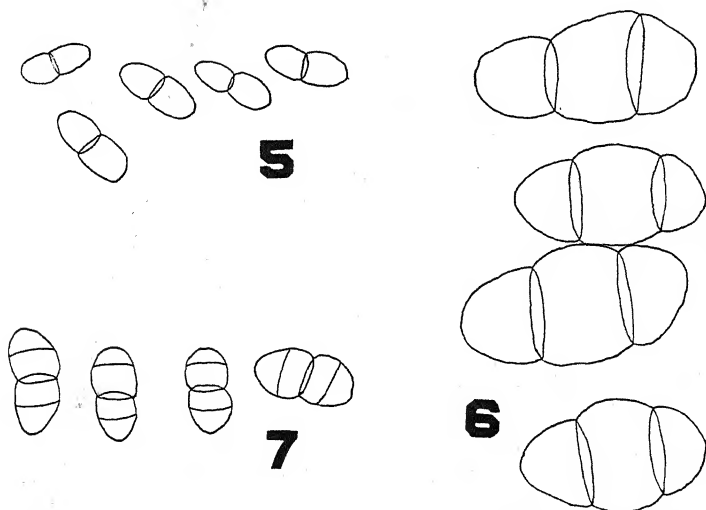
FIG. 5. Spores of *Dimerium alpinum.* FIG. 6. Spores of *Exosporium pedunculatum.* FIG. 7. Spores of *Hysterium acuminatum* var. *alpinum.*

15. **Dimerium alpinum** W. B. Cooke, sp. nov. Mycelio amphigeno, atrobrunneo, ex hyphis repentibus, ramosis, $3.5–10.8 \mu$ diam.; peritheciis ovoideis, membranaceis, non papillatis, levibus, atris; ascis cylindraceis, $43.5–58 \times 8.7–10.2 \mu$, aparaphysatis, octosporis; ascosporis uniseriatis vel biseriatis, ellipsoideis, 1-septatis, medio constrictis, brunneis, $11.6–13.2 \times 4.35–5.8 \mu$.

Hab. ad folia *Pentstemonis menziesii* var. *davidsonii,* 3000 m., Mt. Shasta, Siskiyou Co., Calif., Jul. 30, 1946. W. B. Cooke **18314.**

Perithecia black, without beak, in a dense mycelial mat, $200–250 \mu$ in diameter; hyphae of subiculum black, with thick cuticle, $3.5–10.8 \mu$ in diameter, loosely interwoven, well branched, septate, opaque, sometimes densely encrusted with foreign matter apparently caught in the gelatinous cuticle which is brown in color; asci 43.5–

58 × 8.7–10.2 μ; ascospores brown, 2-celled, 4.35–5.8 × 11.6–13.2 μ, constricted at the septum, uniseriate to biseriate, thick-walled, lower cell slightly smaller than upper cell in some asci.

Forming black crusts usually on lower surface of leaves of *Pentstemon menziesii* var. *davidsonii* (Greene) Piper, sometimes blackening whole plants or portions of plants which grow mat-like in protection of rocks in the White Bark Pine and Alpine Zones, Mt. Shasta, Siskiyou Co., Calif. **Type collection**: W. B. Cooke **18314**, July 30, 1946, 9000 ft., Mt. Shasta. Other collections from the southwest side of Mt. Shasta between 9000 and 10,000 feet in the White Bark Pine and Alpine Zones include W. B. Cooke numbers *8601*, July 28, 1937; *10252*, July 28, 1939; *15711*, Aug. 14, 1941; and *20479*, Aug. 28, 1947.

Collections of material of this species made prior to 1946 and in 1947 were mostly immature. The 1946 collection showed mature ascus and spore characters well. If it seems peculiar that a sooty mold should grow at such high altitudes, it should be remembered that moisture and relative humidity are very high under the snow pack which covers the higher slopes of the mountain for more than six months of normal years. Temperature conditions under the snow pack are not well known but are such that some fungus growth is permitted during the existence of the pack. The moisture and humidity conditions are high enough to satisfy the requirements of large numbers of "micromycetes," most important of which are the brown felt or smothering fungi, *Neopeckia coulteri* on pines and *Herpotrichia nigra* on firs and hemlock.

16. **Cryptosporella acerina** L. E. Wehmeyer, sp. nov. Stromatibus sub cortice nidulantibus discoideis idque pustulatim elevatibus, discis densis, atris, rotundis vel ellipticis erumpentibus; peritheciis 5–10, in cortice immersis, globosis vel elongatis, atro-brunneis, 300–400 × 200–350 μ diam.; ostiolis elongatis, 100–200 μ diam., in discum per peridermium erumpentibus; ascis numerosis, clavatis, basibus deciduis, tenuibus, 45–70 × 12–14 μ, octosporis; ascosporis biseriatis, unicellularibus, hyalinis vel late fusoideis, 10–12.5 × 4.5–5 μ.

Hab. in cortice ramulorum emortuorum *Aceris glabri,* 2000 m., Mt. Shasta, Siskiyou Co., Calif., Aug. 8, 1947. W. B. Cooke **20413**.

On surface as longitudinally seriate, crowded, rounded or elliptic, black, wrinkled or rugose discs which are erumpent through longitudinal ruptures of the periderm. These discs have a tough, rubbery texture, the edges are emarginate, raised, curled inward or

wrinkled. The discs may or may not contain several short-cylindric, punctate ostioles. The disc originates just beneath the periderm as a parenchymatic mass of dark brown, thick-walled, coarse-celled ectostroma which ruptures the periderm. The perithecia, which are formed in clusters of 5–10 beneath the discs, in the bark cortex, are $300–400 \times 200–350\,\mu$ in diameter, globose or radially elongate from crowding, and have stout ostiolar necks, $100–200\,\mu$ in diameter, which penetrate the overlying ectostroma. The walls of the perithecia and ostioles are $15–30\,\mu$ thick and consist of an outer layer of coarse, dark brown, compressed parenchyma cells and a thin layer of light-colored finer hyphae. Asci numerous, clavate, soon deciduous at the base and free in the perithecium, thin-walled with a refractive ring in the apex, $45–70 \times 12–14\,\mu$. Spores biseriate, one-celled, hyaline, ellipsoid to broad fusoid, $10–12.5 \times 4.5–5\,\mu$.

Type collection: on *Acer glabrum* Torr., Sisson Southern Trail Spring, 6000 ft., Mt. Shasta, Aug. 8, 1947, W. B. Cooke **20413**.

This species differs from all other described species of *Cryptosporella* in the large black erumpent discs and in the smaller spores. There are evidences of cavities in the upper portions of the ectostroma, in which conidia may have been formed in the early development of this tissue.

The writer is indebted to L. E. Wehmeyer for studying this and the following species, for preparing the English diagnoses and the notes and the accompanying illustrations of these two species.

17. **Massarinula lignorum** L. E. Wehmeyer, sp. nov. Peritheciis parenchymaticis, crassis, atris, laxe gregariis, in ligno immersis, $600–1000 \times 300–500\,\mu$; ostiolo minuto, papillato; ascis late-clavatis, $105–125 \times 20–25\,\mu$; paraphysibus numerosis, filiformibus, persistentibus; ascosporis biseriatis, hyalinis, fusoideo-ellipsoideis, plus minusve inaequilateralibus vel curvatis, 1-septatis, 4-guttulatis, medio-constrictis, $35–44 \times 10–12\,\mu$, apicibus contractis, plus minusve obtuse rotundatis.

Hab. in ramulis emortuis *Aceris glabri,* 2000 m., Mt. Shasta, Siskiyou Co., Calif., Aug. 8, 1947. W. B. Cooke **20403**.

On surface as rather thickly scattered, circular to elliptic, black pustules with a minute, papillate, central ostiole. Perithecia flattened ellipsoid, $600–1000 \times 300–500\,\mu$, buried in the surface layers of the wood and erumpent through the ruptured overlying fibers. Perithecial walls of very coarse, black-walled parenchyma, thin $(20–30\,\mu)$ below, much thicker $(50–100\,\mu)$ on the upper exposed surface. Asci broadly clavate, wall somewhat thickened above, $105–125 \times 20–25\,\mu$, imbedded in a mass of filiform, hyaline, per-

sistent paraphyses. Spores biseriate, hyaline, fusoid-ellipsoid, somewhat inaequilateral or curved, two-celled but 4-guttulate, possibly becoming 4-celled at full maturity, constricted at the central septum, ends tapered and somewhat bluntly rounded, 35–44 × 10–12 μ.

Type collection: on *Acer glabrum* Torr., near Sisson Southern Trail Spring, 6000 ft., Mt. Shasta, Aug. 8, 1947, W. B. Cooke **20403.**

There exists a large group of species, all of which are very similar in spore form and perithecial structure and to which the species just described belongs. They have fusoid, hyaline, spores with a characteristic "biconic" form, strongly constricted at the middle, swollen on both sides of this septum and tapered toward the ends, usually inaequilateral or curved. These spores are usually 2-celled at first then 4-guttulate and then 4-celled. In some cases they may turn brown at extreme maturity. They may or may not have a gelatinous envelope, which in the writer's opinion is unimportant but it is used as a diagnostic character of the Massariaceae. The color and septation of the spores vary with maturity and with the species, but it is often difficult to distinguish between these two factors because of overlapping types. As a result, these fungi, though closely related, are to be found in a number of genera. They are related on the one hand to certain species of *Didymella* and the genus *Massarinula* in the Massariaceae and on the other hand to certain species of the genus *Metasphaeria* and *Massarina*.

The perithecia have a structure similar to that common in the Massariaceae, with numerous persistent paraphyses. The fungus here described is very similar to *Massarina eburnioides* Sacc., *M. pomacearum* Höhn. and *M. corni* (Fckl.) Sacc. Höhnel (Ann. Myc. **15**: 361. 1917) has already noted the similarity of these species and their relation to *Metasphaeria,* when the gelatinous envelope about the spore is lacking. *Massarinula lignorum* has the same large spores, but these have no gelatinous envelope and they remain, for the most part, two-celled. Although they possess four large guttulae, and a few cases were seen in mounts in Amann's solution where the spore protoplast seemed to be four-parted, most spores were definitely two-celled as seen in this medium.
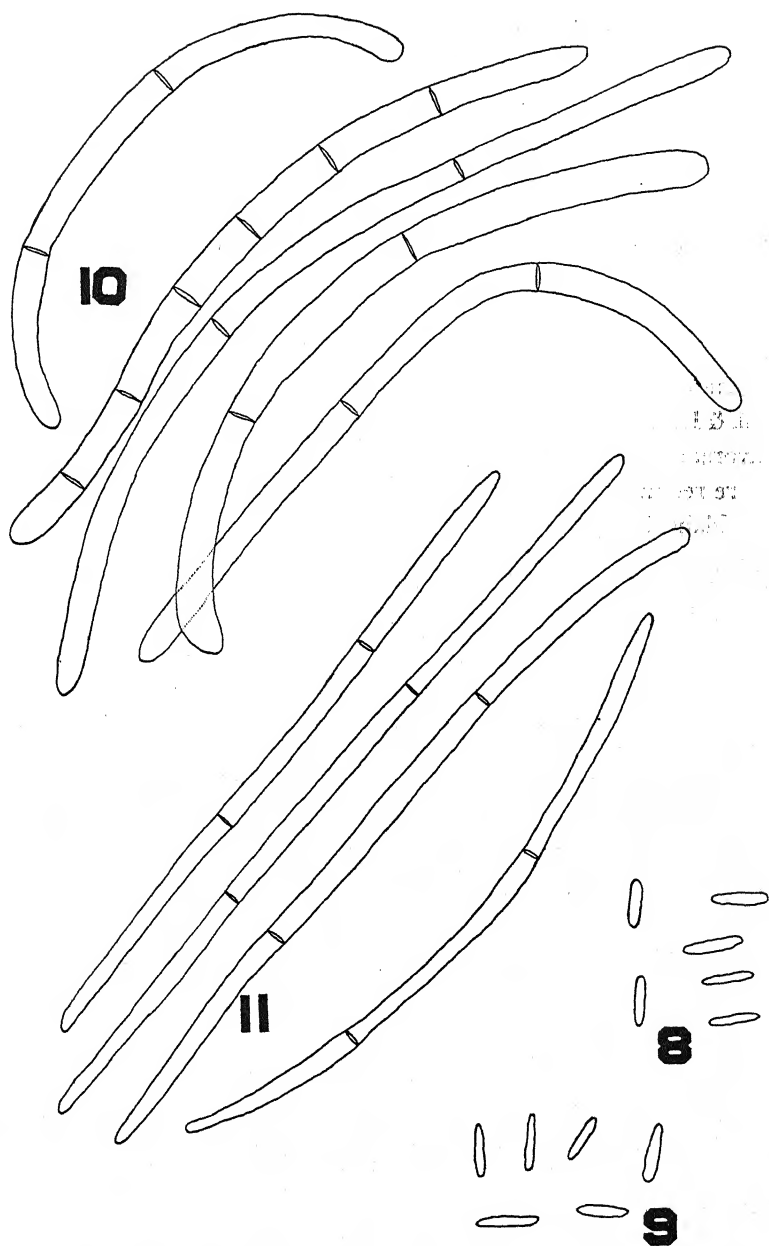
FIG. 8. Spores of *Phyllosticta crustosa*. FIG. 9. Spores of *Hainesia borealis*. FIG. 10. Spores of *Cylindrosporium veratrinum*. FIG. 11. Spores of *Cylindrosporium smilacinae*.

B.   Some species associated with Sambucus

On July 25, 1946, the writer collected at Bear Springs, 5000 ft., Mt. Shasta, Siskiyou Co., Calif., material identified by comparison with material distributed from Northport, Washington, by Science Service, Ottawa, as *Exosporium sambuci* Tracy & Earle.   The Mt. Shasta specimen was distributed as no. *208* in the "Mycobiota of North America."

Recently in checking over material in that part of the C. V. Piper collections housed in the Herbarium of the Botany Department of the State College of Washington, specimens were found of *Brachysporium puccinioides* E. & E. and *Brachysporium pedunculatum* Ell. & Ev. from Pullman, Washington.   These specimens compared favorably with the material from Mt. Shasta, Northport, and a more recent collection from the top of the White Bird Grade, 4400 ft., Idaho Mountain, Idaho Co., Idaho, W. B. Cooke *23807,* June 12, 1948.

In order to clarify the status of these specimens material of *Exosporium* and *Brachysporium* species on *Sambucus* species was kindly loaned by B. B. Kanouse, University of Michigan; H. M. Fitzpatrick, Cornell University; D. P. Rogers, New York Botanical Garden; J. A. Stevenson, Mycological Collections, Beltsville, and G. W. Martin, State University of Iowa.   In addition to specimens of these species, material of *Heterosporium* and *Helminthosporium* was obtained.   It was thought that since *Brachysporium* had been confused with *Exosporium* it was possible that some collections filed under these genera might belong to this complex.

Exosporium was first described by Link (Mag. Ges. Naturf. Fr. Berlin **3**: 9.   1809).   It was used again by Link in 1825 in Vol. 6, part 2, of Wildenow's 4th edition of Linnaeus' Species Plantarum.   Fries reduced the species of *Exosporium* to synonymy in *Helminthosporium* since he considered these species as only stromatic members of this genus.   If it is noted below how later workers have confused *Brachysporium,* a segregate of *Helminthosporium,* with *Exosporium,* one can see how Fries could have placed *Exosporium* in *Helminthosporium.*

In 1869, Fuckel (Symb. Myc. p. 372) applied the name *Cryptocoryneum* to fungi of this type.   Lindau, in Rabenhorst's Krypto-

gamen Flora, ed. 2, Vol. 9, p. 362, 1910, used *Exosporium* and indicated that this was in accord with von Höhnel's treatment. In 1923, von Höhnel accepted both genera and added *Phanerocoryneum* to the group. He distinguished them by assigning to *Exosporium* erumpent, cartilaginous species, to *Phanerocoryneum* species which were superficial, with longish, loosely arranged conidia, and to *Cryptocoryneum* superficial species with long, cylindric, many-celled conidia which occur in dense bundles. Clements and Shear, Genera of Fungi, 1931, indicate that the difference between *Cryptocoryneum* and *Exosporium* is that in the former the sporodochia are superficial, in the latter they are erumpent.

EXOSPORIUM SAMBUCI Tracy & Earle. In 1898 Tracy and Earle distributed as No. *1104* in their "Plants of Colorado" under this name a fungus collected on *Sambucus melanocarpa* along the La Plata River at 10,000 feet, Durango Co., Colorado, by C. F. Baker, F. S. Earle and S. M. Tracy. In this material the sporodochia are visible as black elliptical spots under splits in the bark which may have originated at the lenticels. The sporodochia develop on or below the cambium. The lesions are 0.5–3 × 0.1–0.5 mm. They may eventually become confluent although this is not particularly evident in the two specimens of the type collection from the New York Botanical Garden. The large size is attributed to the large sporodochium rather than to the confluence of more than one sporodochium. The sporophores are densely packed together, 5–6 μ in diameter, septate, yellowish, and are often deciduous, remaining attached to the spores, even after rough treatment in making crush mounts. The conidia are ovate to obovate, yellow-brown, usually 3-septate (although 2-septate spores are seen in every mount), usually constricted at the septa, in spite of the fact that in the original description it is stated that they are not constricted, and measure 40–44 × 17–20 μ.

Brachysporium sphaerocolum F. E. Clements, nom. nud. Material assigned to this species was collected by F. E. and E. S. Clements at 2500 m. (7500–8000 ft.) in Larkspur Dell, Colorado, on July 11, 1905. The specimen was obtained from *Sambucus microbotrya* in a *Picea-Pseudotsuga* Association. It was distributed in Clements' "Cryptogamae Formationum Coloradensium,"
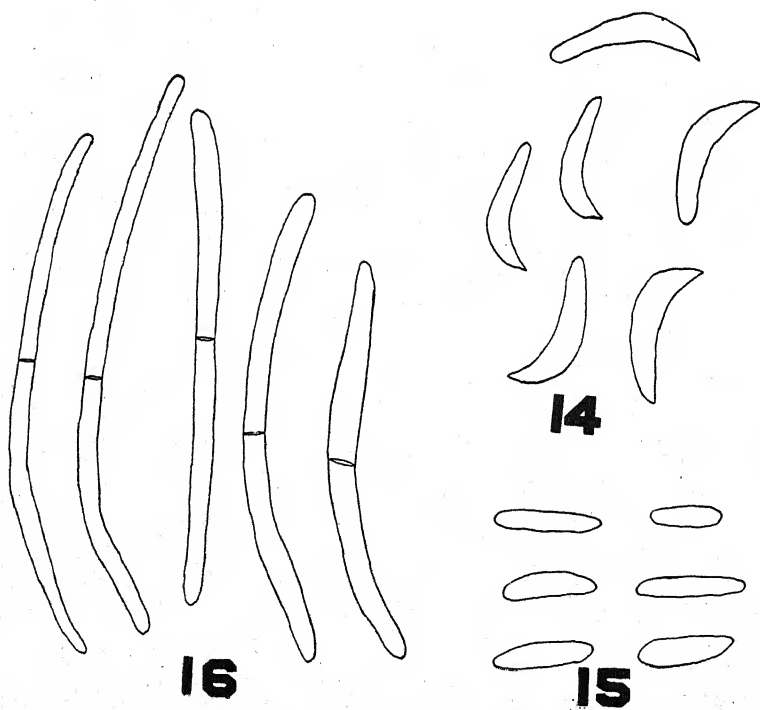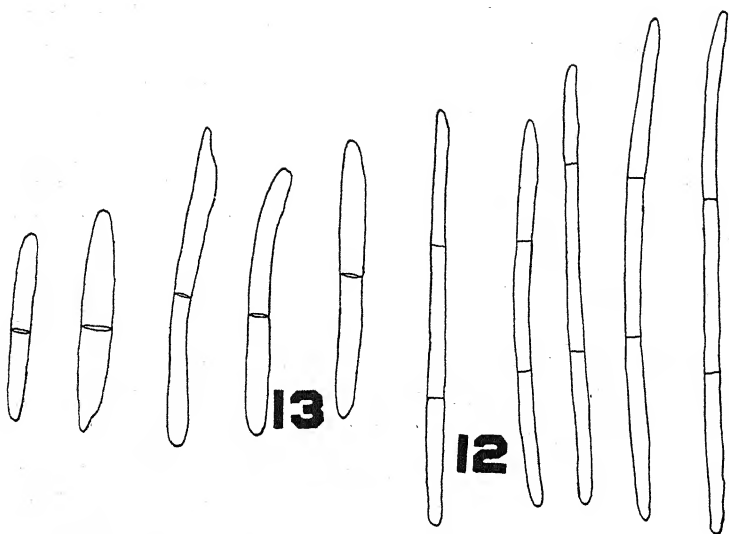
in which it was not described, and was loaned to me by Mr. Stevenson. The label of this specimen merely contains habit and habitat information in Latin. Microscopic examination indicates that this specimen is *Exosporium sambuci* Tracy and Earle. Thus two specimens are on record of *Exosporium sambuci* and both are from Colorado.

BRACHYSPORIUM PUCCINIOIDES Ell. & Ev. C. V. Piper collected material at Pullman, Whitman Co., Washington, W.S.C. *170463,* which Ellis assigned to this species. However, according to a note on the type packet at the New York Botanical Garden, Ellis and Anderson already had assigned this name to a species of *Macrosporium*. Thus Ellis changed the name to *Brachysporium pedunculatum* Ell. & Ev. Piper's specimen was distributed to several herbaria before the correction was made. The earlier name is a *nomen nudum* and should be used only in synonymy with the following species:

Exosporium pedunculatum (Ell. & Ev.) W. B. Cooke, comb. nov.
Brachysporium pedunculatum Ell. & Ev., Proc. Acad. Phil. 1895, p. 440. 1895.
Brachysporium puccinioides Ell. & Ev. in herb., *nomen nudum*.

This species is based on material collected by C. V. Piper on March 24, 1894, at Pullman, Whitman Co., Wash., on dead branches of *Sambucus glauca* Nutt. Piper's specimen, the type, was assigned the number *316,* and duplicates have been observed from the New York Botanical Garden, Mycological Collections, and the Herbarium of the State College of Washington, *170463*.

This species, too, has spores which are produced on sporodochia. These structures, in dry specimens, are hard, black, sclerotic masses. They appear, in microscopic examination, as rather large *Puccinia* pustules. (This accounts for the first name assigned the species by Ellis.) The sporodochium is produced on or under the cambium and breaks through the bark at lenticels, at weak points in the bark, or in elongate linear areas in the internodes. These sporo-

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FIG. 12. Spores of *Septoria lunelliana.* FIG. 13. Spores of *Ramularia claytoniae.* FIG. 14. Spores of *Selenophoma linicola.* FIG. 15. Spores of *Ramularia pentstemonis.* FIG. 16. Spores of *Septoria aromatica.*

dochia may become confluent. Upon making fresh water-mounts
the sporophores may become detached along with the spores, mak-
ing them appear "pedunculate." The sporodochia in the Revel-
stoke collection measure 0.25–0.5 mm. in diameter. Confluence
may increase this size to a half centimeter or more in length.
The conidiophores are $12–15 \times 6–7 \mu$, septate, yellowish. The
spores are produced terminally. The conidiospores are $22–30 \times$
$12–15 \mu$ in the type collection, $25–36 \times 14.5–18 \mu$ in the Revelstoke
collection, and the other collections examined have spores of similar
or intermediate dimensions. The spores are two-septate, brown
to dark brown, usually constricted at the septa, with a swollen cen-
tral cell which gives them a blunt fusiform to barrel shape. Some
of the spores are flattened on one side and this, with the swollen
central cell, gives the spore a *"Curvularia"* aspect. The original
description, and Ellis' notes on the type packet at the New York
Botanical Garden, indicate that Ellis did not notice the constric-
tions at the septa. All the spores observed were two-septate.
In addition to the Pullman specimen, specimens have been examined
from Revelstoke, 1500 ft., British Columbia, collected Sept. 25, 1930
by J. R. Hansbrough *158* (NYBG) ; Northport, Pend Oreille Co.,
Wash., Sept. 24, 1929, A. W. McCallum, distributed by Herbarium,
Division of Botany, Ottawa, Canada (Myc. Coll., U. Mich., U.
Calif.) ; Cedar Mt., Latah Co., Idaho, July 1898, C. V. Piper *691;*
Herb. St. Coll. Wash. *170295;* Mt. Idaho, Idaho Co., Idaho
(WBC) ; Mt. Shasta, Siskiyou Co., Calif. (WBC, Mycobiota of
North America *208*) ; and San Antonio Canyon near Claremont,
Los Angeles Co., Calif. Pacific Slope Fungi, dist. by C. F. Baker,
collected Oct. 10, 1903 by C. F. Baker (NYBG). All have the
same microscopic features although there is some variation in size
and degree of confluence of the sporodochia.

Brachysporium is a member of the Dematiaceae and thus is
non-stromatic or moniliaceous in fruiting habit. Since *Exosporium*
has sporodochia it is placed in the Tuberculariaceae. Since ma-
terial of *Exosporium pedunculatum* Ell. & Ev. in all mounts shows
spores produced on sporodochia it should be placed in the Tu-
berculariaceae rather than in the Dematiaceae. At present, *Exo-
sporium* is the best genus in the Tuberculariaceae in which to place
it. It differs from *Exosporium sambuci* Tracy & Earle, known so

far only from two Colorado collections, in its smaller, three-celled spores.

Link established the genus *Helminthosporium* in 1809. Persoon in 1822 used it in essentially the same sense. The concept of the genus or its description has become enlarged until today it is used in the sense of Lindau, Vol. 9, ed. 2, of Rabenhorst's Kryptogamen Flora. Dreschler (Graminicolous species of *Helmintho-*
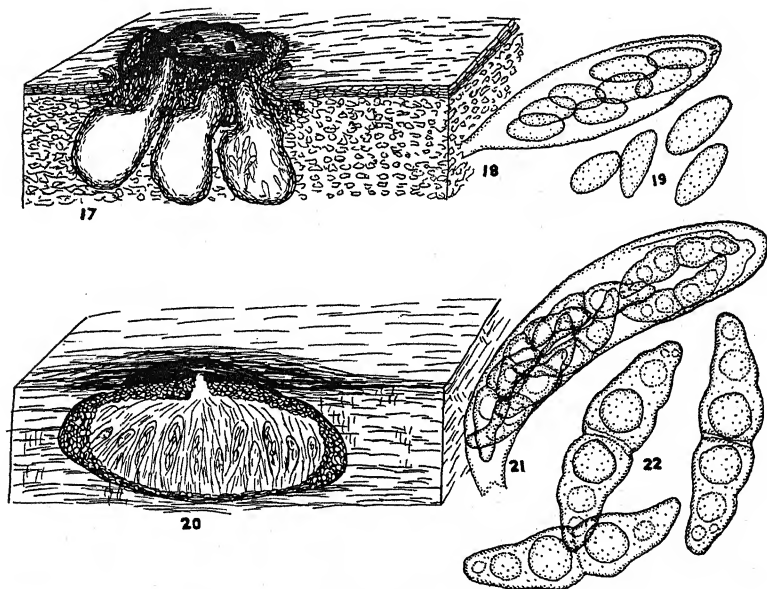
Figures 17–22 drawn by L. E. Wehmeyer.

Fig. 17. Section through perithecial stroma of *Cryptosporella acerina.* Fig. 18. Ascus of *Cryptosporella acerina.* Fig. 19. Ascospores of *Cryptosporella acerina.* Fig. 20. Section through perithecium of *Massarinula lignorum.* Fig. 21. Ascus of *Massarinula lignorum.* Fig. 22. Ascospores of *Massarinula lignorum.*

sporium, Jour. Agr. Res. 24: 641–739. 1923) indicates that *Brachysporium* Sacc. is merely a repository for short-spored species, and *Napicladium* Thüm. is only a repository for tapered-spored species, and that both are only variations of *Helminthosporium* which are not constant and for which various intergradations can be demonstrated. In both Drechsler's and Gilman's (Manual of Soil Fungi, Ames, 1944) treatments the concepts out-

lined by Lindau are followed.   That is, the conidiophores are genic-
ulate and produce multiseptate dark spores acrogenously or, more
usually and in mature specimens, acropleurogenously.   The co-
nidiospores are smooth.

Heterosporium was used first by Klotzsch in Herb. mycol. n. 67
and 69, 1832, for a phragmosporous dematiaceous fungus with
rough spores.   It was also used in this sense by M. C. Cooke
(Grev. II. 122.   1877).   While it is not so indicated in Lindau's
description, the illustrations in the accompanying specific descrip-
tions show acropleurogenous, geniculate conidiophores so that this
fungus is essentially a repository for species of fungi like *Helmin-
thosporium* with roughened spores.   Lindau indicates that *Hetero-
sporium* Klotzsch ex Cooke is mostly parasitic while *Helmintho-
sporium* is mostly saprobic.   This is not a usable delimitation
since a large number of species of *Helminthosporium* are parasitic.

Dendryphiella was erected by Bubak & Ranojevic (N. Ranojevic,
Dritter Beitrag zur Pilzflora Serbiens, Ann. Myc. **12**: 393–421.
1914) to accommodate species of phragmosporous dematiaceous
fungi whose conidia are roughened and produced acropleurogen-
ously on geniculate conidiophores.   It was based on *Helmintho-
sporium interseminatum* Berk. & Rav.   Berkeley included the spe-
cies in *Helminthosporium*.   Probably he did not consider the
roughness on the spores as sufficient for generic segregation.
Dendryphiella is plainly a synonym of *Heterosporium*.

HELMINTHOSPORIUM INTERSEMINATUM Berk. & Rav.   This
species is occasionally found as a brown-black to purple-black fungus
on dead herbaceous or woody stems.   It is poorly represented in
herbaria but specimens on *Sambucus* and *Phytolacca* have been ex-
amined and it has been reported on *Humulus* and *Anthriscus*.
That it is widespread in eastern North America is indicated by the
following locations from which material has been observed: New-
field, New Jersey (BPI, Cornell); Auburn, Lee Co., Alabama
(Cornell); Tuskegee, Alabama (BPI); Lancaster, Fairfield Co.,
Ohio (BPI); Fayette Co., West Virginia (BPI); and near Iowa
City, Iowa (St. Univ. of Iowa).

The felty area on the dead sticks may be sharply limited or have
indefinite limits.   It may be associated with other fungi but no re-
lationship between these fungi and its life cycle is indicated.   Upon

microscopic examination one finds an intricately interwoven mass of branched hyphae which stand erect from the substratum. No sterile hyphae were seen, nor are any described by other workers. The conidiophores may range from 100 to 500 μ long by 6 to 8 μ in diameter. They are simple or branched and when branched the branches become interwoven to form the observed felt. Near each septum may be produced 1–4 spores giving the conidiophores a nodulose or a geniculate appearance. Usually only one spore is produced but the scars of 2, 3, or 4 spore-production points are not infrequent. At the tip of the conidiophore spores also are produced so that the spores are produced acropleurogenously. The conidiophores are smooth to minutely roughened toward the tip.

The spores are usually four-celled although there may be four septa in some cases. They are roughened by fine verrucae. While the type description indicates that the spores are 20–22 μ long, in a collection by G. F. Atkinson at Auburn, Alabama, they are 21.8–25.2 × 4–5 μ, a measurement characteristic of other specimens examined.

Earlier writers indicate that *Helminthosporium vimineum* B. & C. var. γ and *Dendryphium nodulosum* Sacc. are synonyms.

HETEROSPORIUM SAMBUCI Earle. Material assigned to this species by Earle was collected by L. M. Underwood (NYBG) on Mar. 13, 1896, at Auburn, Lee Co., Alabama. Its characters agree well with those of *Helminthosporium interseminatum* and it was placed in synonymy with that species by Atkinson.

Dendryphiella interseminatum (Berk. & Rav.) Bub. & Ran. As indicated above this combination was made to accommodate this rough-spored species of *Helminthosporium* with geniculate conidiophores on which the spores are produced acropleurogenously. Since these conditions are all met by *Heterosporium*, this binomial should be placed in synonymy with the following.

HETEROSPORIUM INTERSEMINATUM (Berk. & Rav.) Atk.

Helminthosporium vimineum B. & C. var. γ

Helminthosporium interseminatum Berk. & Rav.

Dendryphium nodulosum Sacc.

Heterosporium sambuci Earle

Heterosporium interseminatum (Berk. & Rav.) Atk.

Dendryphiella interseminata (Berk. & Rav.) Bub. & Ran.

This combination was made in "Some fungi from Alabama," Bull. Cornell Univ. (Science) 3(1): 1–50. June, 1897. Material on which this combination was based is housed at Cornell University and was collected in 1891 at Auburn, Lee Co., Alabama.

The writer wishes to acknowledge the assistance of Lee Bonar, Dept. of Botany, University of California, and L. E. Wehmeyer, Dept. of Botany, University of Michigan, in identifying the specimens described above; Donald P. Rogers, New York Botanical Garden, for checking the Latin diagnoses and reading the manuscript; the several people listed above for the loan of specimens; and C. G. Shaw, State College of Washington, for checking the manuscript.

BOTANY DEPT.,
    STATE COLLEGE OF WASHINGTON,
        PULLMAN, WASHINGTON

STUDIES ON ROCKY MOUNTAIN FUNGI—I [1]

W. G. Solheim [2]

This paper is the first in a proposed series in which the author plans to publish descriptions of new species and notes on other fungi of special interest. In 1934 the author started issuing a set of fungi entitled "Mycoflora Saximontanensis Exsiccata." Publications covering these issues have appeared in the "University of Wyoming Publications." In these several new species have been described. Through this new series wider circulation will be had for the descriptions of the new species.

In this paper nine species are described as new. All of these will be included in the fifth century of the exsiccata. The present distribution of the set is: Rocky Mountain Herbarium of the University of Wyoming, Farlow Herbarium of Harvard University, Arthur Herbarium of Purdue University, Herbarium of the New York Botanical Garden, Herbarium of the Bureau of Plant Industry, Herbarium of the Department of Plant Pathology of Cornell University, Herbaria of the Universities of California, Colorado, Illinois, Michigan, Tennessee, Wisconsin, Herbarium of Ohio State University, Cryptogamic Herbarium of the University of Toronto, Herbarium of Dr. F. Petrak, Herbarium of the Directorate of Plant Protection, Quarantines and Storage of India, and the author's private herbarium. A set was sent to the late Dr. H. Sydow but this was no doubt destroyed.

Anthostomella ratibidae sp. nov.

Maculis amphigenis, elongatis, usque ad 25×1 mm., nigris: peritheciis innatis, dispersis, globosis, brunneo-nigris, 175–300 μ, per epidermidem nigrifactam obtectis, epidermide in papillas elevata; ostiolo fere plano, 20–36 μ: ascis 8-sporis, cylindricis, 87–104 $\times$ 12.2–15.8 μ: sporidiis monostichis, oblongo-ellipsoideis, 12.2–16 $\times$ 7.5–8.7 μ, olivaceis, 1–2-guttulatis: paraphysatis.

Specimen typicum in foliis et caulibus *Ratibidae tagetis* (James) Barnhart (Compositae), Cienega Canyon Recreational Area, Sandia Mountains, Albuquerque, Bernalillo County, New Mexico, Amer. Bor., 24 Octobri, 1948, legerunt W. G. & Ragnhild Solheim, sub numero **2323**.

Spots amphigenous, elongate, up to 25 × 1 mm., black: perithecia innate, scattered, globose, brownish-black, 175–300 μ, covered by the blackened epidermis which is papillately raised over the perithecia; ostiole almost plane, 20–36 μ: asci 8-spored, cylindrical, 87–104 × 12.2–15.8 μ: spores uniseriate, oblong-elliptical, 12.2–16 × 7.5–8.7 μ, olivaceous, 1–2-guttulate: paraphysate.

On leaves and stems of *Ratibida tagetes* (James) Barnhart, Cienega Canyon Recreational Area, Sandia Mountains, Albuquerque, Bernalillo County, New Mexico, Oct. 24, 1948, W. G. & Ragnhild Solheim No. *2323* (*type*) (Myc. Sax. Exs. No. *428*).

Mature asci are rather rare in this specimen. The ascospores which are first hyaline and then olivaceous may possibly be much darker in more mature specimens.

Lophionema apoclastospora sp. nov.

Peritheciis dispersis vel subgregariis, leniter immersis, nigris, 500–1000 μ altis, ad basem depresso-sphaeroideis, 145–506 μ latis, superna parte prominente, fortiter in latera compressa, 115–318 μ latis, parte basali subinde circumdata mycelio brunneo et araneoso excrescente: mycelio regulari, atrobrunneo; hyphis 3–4 μ crassis, saepe fila parallelium vel intertextarum hypharum formante: paraphysibus numerosis, hyalinis, filiformibus, circa 1.2 μ crassis: ascis 8-sporis, anguste cylindrico-clavatis, ad basem aliquantulum attenuatis, aliquantulum flexuosis, 390–540 × 8.7–12.1 μ: sporidiis cylindraceis, utrimque rotundatis, multiseptatis, aliquando constrictis, olivaceobrunneis, 300–470 × 2.3–3 μ, in segmenta derumpentibus; segmentis 7–31.3 μ longis, continuis vel 1–4-septatis.

Specimen typicum in caulibus emortuis et decorticatis *Salicis* sp. (Salicaceae), Libby Creek prope castra viae, Medicine Bow Mountains, Albany County, Wyoming, Amer. bor., 27 Junii, 1942, legit W. G. Solheim, sub numero **2025**.

Perithecia scattered or subgregarious, slightly immersed, black, 500–1000 μ high, basal portion depressed spherical, 145–506 μ wide, upper portion prominent, strongly compressed laterally, 115–318 μ wide, basal portion at times surrounded by brown, cobwebby, mycelial outgrowths: mycelium regular, dark brown; hyphae 3–4 μ diam., cells several times longer than broad, septa indistinct, frequently forming strands of parallel or interwoven hyphae: paraphyses numerous, hyaline, filiform, about 1.2 μ diam.: asci narrowly cylindrical-clavate, attenuated toward the base, somewhat flexu-

ous, 390–540 × 8.7–12.1 μ: spores cylindrical, ends rounded, multi-septate, constricted at fairly regular intervals, olive-brown, extending throughout most of the length of the ascus, 300–470 × 2.3–3 μ, breaking up at points of constriction into segments; segments 7–31.3 μ long, continuous or 1–4-septate.

On dead, decorticated stems of *Salix* sp., near road camp on Libby Creek, Medicine Bow Mountains, Albany County, Wyoming, June 27, 1942, W. G. Solheim No. *2025* (*type*) (Myc. Sax. Exs. No. *430*).

Only five species of *Lophionema* have previously been described. A comparison of these and the new species is given in table 1.

TABLE 1

Comparison of the Species of *Lophionema*

Species	Perithecia	Asci	Spores	Segments
vermisporum (Ell.) Sacc.	150–200	150–200× 12–15	75–88×3.5–5	
implexum Ell. & Ev.	about 1/3 mm.	150–160× 8–10	subequal to asci	
bambusae v. Höhn.	0.5–1 mm. wide, 500–700 μ high	to 300×10	300×1.8	6–10
chodati Lendner	150 high× 450 wide	90×12–15	80	6–7
cylindrosporum Kauffman	about 200	about 85× 17–20	60–65×4–5	
apoclastospora Solh.	500–1000 high, 145–506 wide at base, 115–318 wide at top	390–540× 8.7–12	300–470× 2.3–3	7–31

Phyllosticta alpinicola sp. nov.

Maculis indefinitis, superficie superna foliarum brunnescente vel nigro-brunnescente in partibus indefinitis vel toto folio decoloratis, superficie inferiore folii simili sed nigriore, ut videtur, propter copiam pycnidiarum et structurarum similium paratheciis: mycelio subhyalino vel flavo; hyphis tenuibus, 1–3 μ: pycnidiis hypophyllis, dispersis, numerosis, globosis, in lineamento circularibus vel in latera compressis vel irregularibus, pallido-brunneis, 66–132 μ; ostiolo plano vel leniter papillato, 24–45 μ: conidiis bacilliformibus, rectis vel leniter curvatis, hyalinis, 4–7 × 0.9–1.2 μ.

Specimen typicum in foliis vivis *Trifolii parryi* Gray (Leguminosae), latere viae infra Brooklyn Lake, Medicine Bow Mountains, Albany County,

Wyoming, Amer. bor., 16 Augusti, 1930, legit W. G. Solheim, sub numero 50.

Spots indefinite, the upper leaf surface becoming brown to blackish-brown in indefinite sectors or the whole leaflet frequently discolored, lower leaf surface similar but appearing blacker due to the abundance of pycnidia and perithecial-like structures: mycelium subhyaline to yellowish; hyphae fine, $1-3\,\mu$: pycnidia hypophyllous, scattered, numerous, globose, in outline circular to laterally compressed to irregular, light brown, $66-132\,\mu$; ostiole plane or slightly papillate, $24-45\,\mu$: conidia bacilliform, straight or slightly curved, $4-7 \times 0.9-1.2\,\mu$.

On living leaves of *Trifolium parryi* Gray, roadside below Brooklyn Lake, Medicine Bow Mountains, Albany County, Wyoming, Aug. 16, 1930, W. G. Solheim No. *50* (*type*) (Myc. Sax. Exs. No. *454*).

Associated with this fungus are few to many, immature, blackish-brown, perithecial-like bodies.

This species differs from *P. bonansiana* Sacc. in having smaller conidia. Saccardo (Syll. Fung. **25** : 49) gives the dimensions of the conidia as $7 \times 2.5\,\mu$. *P. trifolii* Rich. (Syll. Fung. **10** : 128) has ovoid conidia $2-3\,\mu$ long. *P. medicaginis* (Fckl.) Sacc. has spores $5-10 \times 1.5-2\,\mu$ as represented in a pure culture specimen made by Dr. Lee Bonar, University of California Herbarium No. *568035*. Grove (Brit. Stem- and Leaf-Fungi **2** : 140. 1937) has this species as a synonym of *Sporonema phacidioides* Desm. and gives the dimensions of the conidia as $5-7 \times 1.5-2\,\mu$. The measurements for *P. trifolii-minoris* Unam. (Bol. R. Soc. Espan. Hist. Nat. **29** : 120–121. 1929) are: pycnidia $75.5-83.5 \times 66.5-73.5\,\mu$, conidia $5-7 \times 2.5-3.5\,\mu$. *P. ignatiana* Unam. (Mem. R. Soc. Espan. Hist. Nat. **15** : 348. 1929) is described as having pycnidia $130 \times 140\,\mu$ and conidia $6.5-7 \times 3\,\mu$.

Phyllosticta ragnhildae sp. nov.

Maculis amphigenis, subcircularibus, ellipticis vel elongatis, $2-14 \times 2-5$ mm., aliquando nervulis maioribus limitatis, atrobrunneis vel nigris, margine indefinita, folii textura cingente flavescente: mycelio hyalino; hyphis $2-3.5\,\mu$: pycnidiis amphigenis, dispersis, globosis, aliquando in latera compressis, $87-146 \times 76-111\,\mu$, pallido-brunneis; ostiolo plano, $20-42\,\mu$: conidiis bacilliformibus, rectis vel curvatis, hyalinis, $3.5-5.2 \times 1\,\mu$.

Specimen typicum in foliis vivis *Antennariae pulcherrimae* (Hook.) Greene (Compositae), Happy Jack Picnic Area, Laramie Mountains, Albany

County, Wyoming, Amer. bor., 6 Augusti, 1942, legerunt W. G. & Ragnhild Solheim, sub numero **2093**.

Spots amphigenous, subcircular, elliptical to elongate, 2–14 × 2–5 mm., at times limited by the larger veins, dark brown to black, border indefinite, surrounding leaf tissue becoming yellow: mycelium hyaline; hyphae 2–3.5 μ: pycnidia amphigenous, scattered, globose, at times laterally compressed, 87–146 × 76–111 μ, light brown; ostiole plane, 20–42 μ: conidia bacilliform, straight or curved, hyaline, 3.5–5.2 × 1 μ.

On living leaves of *Antennaria pulcherrima* (Hook.) Greene, Happy Jack Picnic Area, Laramie Mountains, Albany County, Wyoming, Aug. 6, 1942, W. G. & Ragnhild Solheim No. *2093* (*type*) (Myc. Sax. Exs. No. *462*).

P. antennariae Ell. & Ev. has conidia 7 × 3 μ. Associated with this specimen are immature fruiting bodies, probably young perithecia. It is possible that the new species is the spermagonial stage of some ascomycete.

Phyllosticta smilacinae sp. nov.

Maculis amphigenis, elongatis, in maximam partem nervulis maioribus limitatis, 1–5.5 cm. longis, 3–7 mm. latis, pallido-brunneis, brunneis vel brunneo-albis, aliquando partim chlorinis, margine in latera definita, in terminis definita vel indefinita, brunneis vel rufo-brunneis: mycelio sub-hyalino vel diluto flavido-chlorino; hyphis 1.2–3.5 μ: pycnidiis epiphyllis, subinde hypophyllis, numerosis, dispersis, globosis vel leniter in latera compressis, pallidis flavo-brunneis, 49–83 μ; ostiolo in brevem papillam erecto, 14–35 μ: conidiis cylindrico-bacilliformibus, hyalinis, 4.5–7 × 1–1.4 μ, utrimque rotundatis.

Specimen typicum in foliis flavidis *Smilacinae amplexicaulis* Nutt. (Liliaceae), Ouray Picnic Grounds, Ouray, Ouray County, Colorado, 12 Octobri, 1948, legerunt W. G. & Ragnhild Solheim, sub numero **2250**.

Spots amphigenous, elongated, mostly limited by the major veins, 1–5.5 cm. long, 3–7 mm. wide, light brown, brown, to brownish-white, at times partly green, border definite laterally, definite to indefinite terminally, brown to reddish-brown: mycelium sub-hyaline to dilute yellowish-green; hyphae 1.2–3.5 μ: pycnidia epiphyllous, occasionally also hypophyllous, numerous, scattered, globose or slightly compressed laterally, light yellow-brown, 49–83 μ; ostiole short papillate, 14–35 μ: conidia cylindrical-bacilliform, hyaline, 4.5–7 × 1–1.4 μ, ends rounded.

On yellowing leaves of *Smilacina amplexicaulis* Nutt., Ouray Picnic Grounds, Ouray, Ouray County, Colorado, Oct. 12, 1948,

W. G. & Ragnhild Solheim No. 2250 (type) (Myc. Sax. Exs. No. 464).

This species differs from *P. vagans* Pk. in the larger spores and the production of definite spots. In *P. vagans* the pycnidia are recorded as 75–90 μ and the spores $3 \times 1\,\mu$. *P. woronowii* Woronichin is described as having pycnidia 45–75 μ and conidia $3 \times 1\,\mu$. It is probably not distinct from *P. vagans*. The other species of *Phyllosticta* described on *Smilacina* and related genera have ovoid and wider conidia.

Phleospora muhlenbergiae Sprague & Solh. sp. nov.

Maculis coloris - straminei, sine marginibus: pycnidiis dispersis vel subgregariis, innatio-erumpentibus, subcarbonaceis, subglobosis, elliptico-oblongis vel obtuso-lenticularibus, nigro-brunneis, 95–245 $\times$ 65–121 μ; poro irregulariter marginato, qui videtur velut erosus, elongato-elliptico, 45–170 $\times$ 38–104 μ: conidiis subcylindraceis vel anguste obclavatis, apice subacuto, basi vergente in rotundum sed postea obtusa, curvulis vel aliquando rectis, subhyalinis vel pallide chlorino-flavidis, 21–45 $\times$ 2.6–4 μ, 1–2-septatis.

Specimen typicum in foliis vivis *Muhlenbergiae arizonicae* Scribn. (Agrostideae), Oak Flats Picnic Grounds, Santa Catalina Mountains, prope Tucson, Arizona, Amer. bor., 12 Novembris, 1948, legerunt W. G. & Ragnhild Solheim, sub numero **2448**.

Spots straw colored, without borders: pycnidia scattered to subgregarious, innate-erumpent, subcarbonous, subglobose, ellipticaloblong to bluntly lenticular, blackish-brown, 95–245 $\times$ 65–121 μ; pore irregularly margined, appearing as if eroded away, elongate-elliptical, 45–170 $\times$ 38–104 μ: conidia subcylindrical to narrowly obclavate, apex tapering to a softly blunted point, base round-tapering, finally blunt, subhyaline to pale greenish-yellow, curved or at times straight, 21–45 $\times$ 2.6–4 μ, 1–2-septate.

On living leaf blades and sheaths of *Muhlenbergia arizonica* Scribn., Oak Flats Picnic Grounds, Santa Catalina Mountains, east of Tucson, Arizona, Nov. 12, 1948, W. G. & Ragnhild Solheim No. 2448 (*type*) (Myc. Sax. Exs. No. *478*).

The spores of this species are smaller than those of *P. idahoensis* Sprague and *P. graminivora* Sprague & Hardison.

Kabatia fragariae sp. nov.

Maculis nullis vel indefinitis vel definitis et irregularibus; superna superficie folii nigro-punctata, aliquando folii textura cingente flavida vel atrorufa; infera folii textura aliquando paucis et minutis et nigris punctis maculata; textura infra pycnidia epiphylla leniter discolorata vel albido-flavida: mycelio

hyalino; hyphis tenuibus, circa 0.8–1.7 μ: pycnidiis plerumque epiphyllis, subinde hypophyllis, numerosis, dispersis vel gregariis, dimidiatis, disparis coloris atro-brunnei et cinereo-brunnei, marginibus flavis et distincte radiatis, in lineamentis circularibus, subellipticis vel aliquantulum irregularibus, 70–376 × 70–290 μ; poro simplici elongato vel irregulariter furcato et recurvo, cum utraque superficie scutelli exhibente breves, atrobrunneas cellulas papillatas: conidiophoris clavatis, hyalinis, circa 10 × 2.3 μ: conidiis falcatis, apud apicem apiculatis, ad basem attenuatis, hyalinis, 18–28 × 4.5–6.5 μ, 1-septatis.

Specimen typicum in foliis vivis *Fragariae ovalis* (Lehm.) Rydb. (Rosaceae), Happy Jack Picnic Area, Laramie Mountains, Albany County, Wyoming, Amer. bor., 8 Augusti, 1942, legerunt W. G. & Ragnhild Solheim, sub numero **2114**.

Spots none or indefinite or definite and irregular; upper surface of the leaf black dotted or peppered with the pycnidia, at times with the surrounding leaf tissue yellowish to dark red; lower leaf surface at times with a few, minute, black spots, leaf tissue below the epiphyllous pycnidia only slightly discolored or at times becoming whitish-yellow: mycelium hyaline; hyphae fine, about 0.8–1.7 μ in diameter: pycnidia mostly epiphyllous, occasionally hypophyllous and then below those occurring on the upper surface, numerous, scattered or so closely aggregated as to appear coalescent, dimidiate, mottled dark brown and grayish-brown, edges yellow and distinctly radiate, outline circular to subelliptical to somewhat irregular, 70–376 × 70–290 μ, opening by a simple, elongate slit or by irregular, branched slits, the free ends curling back, both surfaces of the scutellum with short, dark brown, papillate cells: conidiophores clavate, hyaline, about 10 × 2.3 μ: conidia falcate, apiculate at apex, tapering toward base, 18–28 × 4.5–6.5 μ, hyaline, 1-septate.

On living leaves of *Fragaria ovalis* (Lehm.) Rydb., Happy Jack Picnic Area, Laramie Mountains, Albany County, Wyoming, Aug. 8, 1942, W. G. & Ragnhild Solheim No. 2114 (*type*) (Myc. Sax. Exs. No. *482*).

A comparison of the species of *Kabatia* is given in table 2.

TABLE 2

Comparison of the Species of *Kabatia*

Species	Pycnidia	Spores
lonicerae (Harkn.) Höhn. = *latemarensis* Bub.	110–180	24–46×6–9
mirabilis Bub.	100–180	33–55×7–11
fragariae Solh.	70–376×70–290	18–28×4.5–6.5

Cylindrosporium corni sp. nov.

Maculis amphigenis, supra conspicuis magis quam infra, subcircularibus angulosis vel irregularibus, minutis, 0.5–2 mm., fumosocinereis, margine angusta, elevata, atra, cingulata cingulis rufopurpureis: mycelio intercellulari, hyalino vel subhyalino; hyphis tenuibus, 0.8–2.5 μ: acervulis epiphyllis, dispersis, subcircularibus, 42–80 μ: conidiophoris hyalinis vel subhyalinis, 6–14 × 2.5–3.5 μ: conidiis filiformibus, ad apicem attenuatis, flexuosis, hyalinis, 45–87 × 1.5–2 μ, 1–5-septatis.

Specimen typicum in foliis vivis *Corni stoloniferae* Michx. (Cornaceae), Six Mile Gap, Platte River, Medicine Bow Mountains, Carbon County, Wyoming, Amer. bor., 7 Septembri, 1948, legit W. G. Solheim, sub numero **2224**.

Spots amphigenous, more conspicuous above than below, subcircular, angular to irregular, minute, 0.5–2 mm., smoky-gray, with a narrow, raised, dark border, surrounded by a reddish-purple zone: mycelium intercellular, hyaline to subhyaline; hyphae fine, 0.8–2.5 μ: acervuli epiphyllous, scattered, subcircular, 42–80 μ: conidiophores hyaline to subhyaline, 6–14 × 2.5–3.5 μ: conidia filiform, tapering upward, flexuous, hyaline, 45–87 × 1.5–2 μ, 1–5-septate.

On living leaves of *Cornus stolonifera* Michx., Six Mile Gap, Platte River, Medicine Bow Mountains, Carbon County, Wyoming, Sept. 7, 1948, W. G. Solheim No. 2224 (*type*) (Myc. Sax. Exs. No. *488*).

The spots are frequently localized in larger discolored areas of the leaf. In view of the fact that many of the spots are independent of these discolored areas it is possible that these discolored areas are not caused by this fungus.

Cylindrosporium saximontanense sp. nov.

Maculis amphigenis, subcircularibus, irregularibus vel angulosis, nervis limitatis, 2–7 mm. longis, brunneis, supra cinerescentibus: marginatis per nervulos supra nigrescentes, infra flavescentes: mycelio intercellulari, subhyalino; hyphis 1.3–3.5 μ: acervulis innatis, nigro-punctatis, epiphyllis, subcircularibus vel leniter ellipticis, 94–160 × 80–115 μ: conidiophoris 8–12 × 3–3.5 μ: conidiis cylindricis vel cylindrico-fusiformibus, rectis vel leniter curvis, subhyalinis vel pallide chlorino-flavis, 28–58 × 3.5–5.2 μ, non septatis.

Specimen typicum in foliis *Populi angustifoliae* James (Salicaceae), Ouray Picnic Grounds, Ouray, Ouray County, Colorado, Amer. bor., 12 Octobri, 1948, legerunt W. G. & Ragnhild Solheim, sub numero **2258**.

Spots amphigenous, subcircular, irregular to angular, vein-limited, 2–7 mm. long, brown, becoming grayish above, bordered by the veins which become blackish above and yellow below: mycelium intercellular, subhyaline; hyphae 1.3–3.5 μ: acervuli

innate, black punctate under a hand lens, epiphyllous, subcircular or slightly elliptical, 94–160 × 80–115 μ: conidiophores 8–12 × 3–3.5 μ: conidia cylindrical to cylindrical-fusiform, straight or slightly curved, subhyaline to dilute greenish-yellow, 28–58 × 3.5–5.2 μ, continuous.

On leaves of *Populus angustifolia* James, Ouray Picnic Grounds, Ouray, Ouray County, Colorado, Oct. 12, 1948, W. G. & Ragnhild Solheim No. 2258 (*type*) (Myc. Sax. Exs. No. *490*).

This differs from *C. oculatum* Ell. & Ev. on this same host in having larger, more irregular spots and epiphyllous acervuli and in having broader, nonseptate conidia.

MINERAL OIL AND PRESERVATION OF FUNGOUS CULTURES

MARY E. STEBBINS AND WILLIAM J. ROBBINS [1]

Buell and Weston [2] reported the use of heavy mineral oil for the preservation of fungous cultures. The fungi covered by their report included few Basidiomycetes. Since we had many of this group in our collection [3] to which the oil method has been applied, it seems desirable to supplement their results by a report on ours. For a review of earlier literature and details of the method, the reader is referred to the article by Buell and Weston.

Beginning March 4, 1947, cultures of 1959 isolations of fungi [4] were transferred to 2 per cent malt agar slants. The tubes were incubated at 25° C. until growth had started (2 days to 1 week) and were then put at 15° C. until the fungi had grown over the surface of the slants (about 2 weeks from the time of inoculation). The slants were not left at 25° C. for 2 weeks because the agar became too dry. The slants were then covered with sterile mineral oil. The oil was Parke-Davis heavy mineral oil, and enough was used in each tube to come about 1 cm. above the top of the agar slant. After they were oiled, all tubes were placed in an upright position at 15° C.

Species of the following genera were treated with mineral oil:

[2] Buell, Caroline B., and William H. Weston. Application of the mineral oil conservation method to maintaining collections of fungous cultures. Am. Jour. Bot. **34**: 555–561. 1947.

[4] These included 853 identified and 100 unidentified species. The balance (1006) were duplicate isolations.

	Number of species		Number of species
ACTINOMYCETES		Calocera	1
Actinomyces	2	Calodon	1
	—	Calvatia	2
PHYCOMYCETES		Ceracea	1
Absidia	2	Claudopus	1
Conidiobolus	1	Clavaria	5
Mucor	2	Clitocybe	8
Phycomyces	2	Collybia	7
Phytophthora	1	Coniophora	6
Pythiomorpha	1	Conocybe	2
Pythium	2	Coprinus	20
Rhizopus	3	Coriolus	1
	—	Corticium	35
	14	Crepidotus	3
	—	Crucibulum	1
ASCOMYCETES		Cyathus	1
Apioporthe	1	Cyphella	1
Ashbya	1	Cytidia	1
Botryotinia	5	Dacryomyces	3
Ceratostomella	19	Daedalea	9
Chaetomium	5	Deconica	2
Ciboria	5	Drosophila	17
Ciborinia	1	Echinodontium	1
Claviceps	1	Eichleriella	1
Cryptodiaporthe	2	Exidia	2
Elsinoë	9	Favolus	2
Endoconidiophora	3	Femsjonia	1
Endothia	1	Fistulina	1
Gibberella	2	Flammula	6
Grosmannia	1	Fomes	42
Lambertella	5	Fomitiporia	3
Massaria	1	Galera	1
Massariovalsa	1	Galerina	1
Melanconis	1	Ganoderma	3
Monascus	1	Gloeocystidium	1
Morchella	1	Gloeotulasnella	1
Neurospora	3	Grandinia	2
Ophiobolus	1	Helicogloea	2
Ophiostoma	1	Hericium	2
Pyrenochaeta	1	Hirneola	1
Rutstroemia	1	Hydnum	10
Sclerotinia	9	Hymenochaete	5
Streptotinia	1	Hypholoma	2
Stromatinia	2	Hypochnus	2
Teichospora	1	Irpex	2
Thielavia	2	Lentinus	6
Xylaria	1	Lenzites	10
	—	Lepiota	1
	89	Leptoporus	1
	—	Lycoperdon	2
BASIDIOMYCETES		Marasmius	6
Agaricus	1	Melanoleuca	1
Agrocybe	2	Merulius	6
Aleurodiscus	7	Mucidula	2
Alnicola	2	Mucronella	1
Armillaria	1	Mycena	3
Auricularia	1	Mycoacia	1

	Number of species		Number of species
Basidiomycetes (Cont'd.)		Botrytis	4
Naucoria	1	Brachysporium	1
Nyctalis	1	Cadophora	2
Odontia	7	Cephalosporium	1
Omphalia	3	Cephalothecium	1
Oxydontia	2	Cercosporidium	1
Panaeolina	1	Chaetomella	1
Panaeolus	5	Chalaropsis	1
Panellus	1	Cladosporium	2
Panus	5	Coccosporium	1
Paxillus	1	Curvularia	4
Pellicularia	3	Dendryphium	1
Peniophora	30	Epidermophyton	2
Phaeolus	1	Fusarium	1
Phallus (Ithyphallus)	1	Gliocladium	4
Phellinus	2	Gliomastix	1
Phlebia	4	Helicoma	1
Pholiota	12	Helminthosporium	1
Pleurotus	9	Heterosporium	1
Pluteus	1	Hormiactella	1
Polyporus	104	Humicola	1
Polystictus	2	Macrosporium	1
Poria	67	Memnoniella	1
Psalliota	1	Metarrhizium	3
Pseudocoprinus	2	Microsporum	4
Ptychogaster	2	Monotospora	1
Radulum	2	Myrothecium	3
Schizophyllum	2	Myxosporium	1
Sebacina	3	Myxotrichella	1
Solenia	1	Nigrospora	1
Sparassis	1	Penicillium	9
Sphaerobolus	1	Pestalotia	4
Spongipellis	1	Phialophora	2
Steccherinum	2	Phoma	1
Stereum	24	Pullularia	1
Stropharia	2	Rhizoctonia	2
Trametes	24	Sclerotium	2
Trechispora	2	Scopulariopsis	2
Tremella	1	Sphaceloma	10
Tricholoma	2	Sphaeropsis	1
Trogia	1	Sporotrichum	2
Tulasnella	2	Stachybotrys	2
Typhula	1	Stemphyllium	3
Ungulina	2	Stysanus	1
Vararia	4	Synsporium	1
Volvaria	1	Tetracoccosporium	1
Xanthochrous	3	Torula	1
	——	Trichophyton	8
	626	Trichosporium	1
	——	Tritirachium	2
Fungi Imperfecti		Verticicladium	1
Achorion	1	Verticillium	1
Acladium	1	Volutella	1
Acremonium	1	Xenosporium	1
Alternaria	1	Zygodesmus	1
Aspergillus	7		——
Botryodiplodia	1		122

All the cultures were examined at intervals and the following observations were made:

Some of the fungi grew better under oil. Among these were species of *Corticium, Epidermophyton, Ashbya, Massaria, Melanconis, Nyctalis, Elsinoë* and *Sphaceloma*. For example, species of *Melanconis, Elsinoë* and *Sphaceloma,* which formed small colonies with limited growth on 2 per cent malt agar, covered the entire slant under oil.

Five types of growth under oil were noted. The numbers refer to the number of isolations in each type.

(1) The mycelium grew appressed to the agar slant. None developed in the oil. (437)

(2) The mycelium grew over the agar slant as in (1) but the mycelial surface was fuzzy. (524)

(3) The mycelium filled the oil but stopped at the surface of the oil or a short distance below it. (145)

(4) A band of mycelium ranging in thickness from less than 1 mm. to 10 mm. formed across the tube directly beneath the surface of the oil and above the top of the agar slant. The oil below this band was free of mycelium. (383)

(5) A narrow band of mycelium (2 mm. or less in thickness) formed at the top of the slant and hung down over the slant with varying amounts of oil free of mycelium above and below the band. (470)

In general, the species of each genus tended to have the same type of growth. However, there were exceptions, and in some instances isolations of the same species differed in type of growth.

The mycelium of a substantial number (153) of the cultures eventually grew up out of the oil. In such cultures evidence of partial drying of the agar slant was observed. The oiled cultures were therefore examined at intervals of six months and additional mineral oil added to those in which the mycelium appeared above the oil.

Pigments from some of the fungi dissolved in the oil. The colors ranged from tan and light yellow to a medium reddish purple, deep red or deep yellow. *Phlebia strigosozonata* and

species of *Elsinoë* and *Sphaceloma* developed pink, orange and red pigments, yellows were observed for species of *Aspergillus, Corticium, Drosophila, Hydnum, Oxydontia, Poria, Tetracoccosporium* and several others (20 genera in all). The reddish purple color was found only with cultures of *Helminthosporium*.

Some of the fungi did not develop their normal color under oil. *Corticium coeruleum* did not form its dark blue pigment, nor did species of *Fusarium* or *Gibberella* become red. *Penicillia* were white, tan or faint olive-green instead of green or blue-green.

Beginning January 21, 1948, transfers were made from the oiled cultures. Bits of mycelium were drained as free from oil as possible by patting them against the inside of the oiled tube and were transferred to fresh 2 per cent malt agar slants. The slants were incubated at 25° C. All the fungi were viable except *Pythium butleri, P. helicoides, Peniophora sambuci,* two isolations of *Sebacina cinerea* and two unidentified species of *Sebacina*. The five latter fungi grew poorly and slowly on malt agar before oiling.

In general, it was our impression that the subcultures from the oiled cultures grew more vigorously than transfers from unoiled cultures.

The cultures oiled in 1947 were again tested for viability in January 1949 after two years under oil. Transfers of *Ashbya gossypii* and *Phallus (Ithyphallus)* sp. failed to grow; both had survived one year under oil. All others grew.

Additional fungi were treated with mineral oil in 1948. Of these, only five genera—*Acanthocystis, Amanita, Armillariella, Eremothecium* and *Rhodopaxillus*—were not included in the original set. All were tested after one year under oil and found to be viable.

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NEW CELLULOSE DESTROYING FUNGI ISOLATED FROM MILITARY MATE-RIAL AND EQUIPMENT

L. M. Ames *

(WITH 42 FIGURES)

Cellulose destroying fungi are valued for their part in the process of reducing plant remains to humus for the enrichment of the soil. The magnitude of this valuable decomposition process is scarcely considered by most people because it is so commonplace and proceeds naturally and continuously. However, these organisms do not distinguish between waste cellulosic materials and the raw or fabricated cellulosic goods of value to human needs. In this latter respect, the destruction of vast quantities of goods by fungi during the recent war focused the attention of military departments on the need of finding means of preserving and lengthening the life and dependability of military equipment. This entailed identifying the organisms concerned as well as prescribing and testing preservative materials. The efforts to this end have been shared by the military departments with civilian government agencies, commercial research establishments, and college laboratories. Although much progress has been made to prolong the life and dependability of cellulosic materials, only a good start has been made to solve the many encountered problems. Many people in industry and government are synthesizing new chemical compounds designed to treat various goods to prevent mildewing. At the same time, many independent and cooperative screening tests are in progress to evaluate old and new fungicides; the more promising are put through many additional exacting evaluation tests. Paralleling these researches, the fungi responsible for mildewing have received considerable attention.

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Many and various kinds of fungi have been isolated from deteriorating equipment at home and in foreign areas; these fungi have been studied both as to their identity and their physical char-
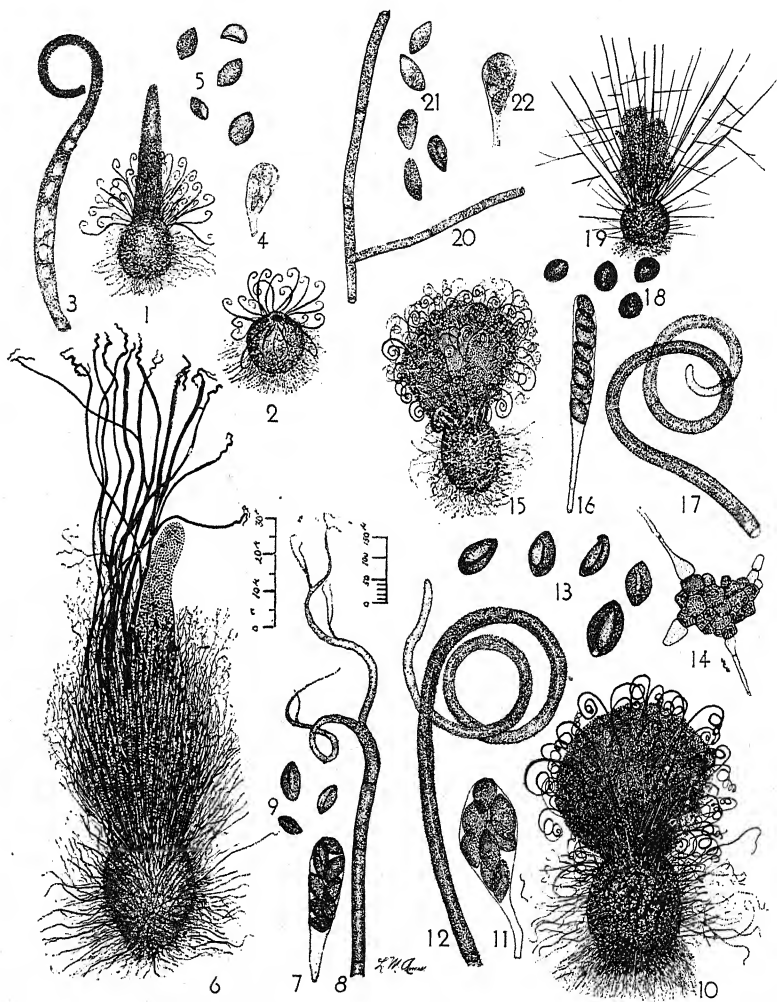
FIGS. 1–22. New species of *Chaetomium*.

acteristics. In the course of isolating and studying fungi from rotting equipment, new organisms, from time to time, have been discovered following which their morphology and growth charac-

teristics were observed. This paper is concerned primarily with nine hitherto undescribed species of mildew-producing fungi belonging to the genus *Chaetomium*. These are illustrated and described below.

Chaetomium turgidopilosum sp. nov.   Figs. 1–5.

Peritheciis pullis, ostiolatis, globosis vel ovatis, 120–140 × 115–135 μ, cirrhis conspicuis provisis et rhizoideis tenellis ad substratum affixis. Pilis lateralibus numerosis, distincte septatis, tenuibus. Pilis terminalibus robustis, rigidis, distincte septatis, basi dilute brunneis supra pallidioribus, medio plerumque plus minusve inflatis, 5–8 μ latis, in apicibus frequenter recurvatis angustatis. Ascis clavatis, octosporis, 20–22 × 9.5–11.5 μ, parte sporifera 15 μ. Ascosporis maturis brunneis, limoniformibus, utrinque leniter apiculatis, 8–11 × 4–7 μ.

Perithecia soiled gray in color, ostiolate, globose to ovate, 120–140 × 115–135 μ, provided with conspicuous cirrhi, attached to the substratum with delicate rhizoids. Lateral hairs numerous, distinctly septate, tapering. Terminal hairs large, rigid, distinctly septate, light brown at the base, lighter in the usually inflated middle section, narrower and darker at the recurved apex. Asci clavate, eight-spored, 20–22 × 9.5–11.5 μ, spore part 15 μ. Mature ascospores are brown, lemon-shaped somewhat apiculated at both ends, 8–11 × 4.7 μ.

Type—isolated from the top of a storage tent. Culture was made by Dr. G. W. Martin and sent to the writer under the designation J-730 APO 929 (A-lff).

This species is easily recognized by the distinctively inflated terminal hairs which are reflexed at their tips.

Chaetomium cristatum sp. nov. Figs. 6–9.

Peritheciis griseis, magnitudinis mediae, ovatis in subglobosis gradientibus, ostiolatis, 250–300 × 175–225 μ, cum rhizoideis brunneis dilutis paratis. Pilis lateralibus copiosis, gracilibus, septatis, apicibus acuminatis. Pilis terminalibus typorum duorum: (a) aliis numerosis septatis, simpliciter vel compositer ramosis, basi 3–4 μ diametro, apicibus subtiliter divisis, peritheciis bysso similibus sedentibus; (b) aliis parvis, longis, atris, basi 6–8 μ diametro, pilos byssinos excedentibus, interdum in madore anastomosantibus quandoque apice ramis parvulis. Ascis clavatis, octosporis, 45 × 12 μ, parte sporifera 28 μ. Ascosporis maturis ovatis, umbonatis vel subapiculatis, olivaceobrunneis, 8–12 × 4.5–6 μ.

Perithecia gray, of medium size, ovate to subglobose, ostiolate, 250–300 × 175–225 μ, attached to the substrate with light brown

rhizoids. Lateral hairs are numerous, slender, septate and gradually decreasing in diameter to the apex. The terminal hairs are of two types: (a) the first are numerous, septate, branched and rebranched, at the base 3–4 μ in diameter and decreasing to thin tips giving the plant a fuzzy appearance; (b) the second are few in number, long and black, at the base 6–8 μ in diameter, extending through and beyond the first type, are occasionally seen anastomosed near the tips which are sometimes abruptly frayed with little branches. Asci clavate, eight-spored, 45 × 12 μ, spore part 28 μ. Ascospores, when mature, are umbonate to subapiculate, olive-brown, 8–12 × 4.5–6 μ.

Type—isolated from paper carton under test in the Tropical Testing Chamber, Fort Belvoir, Virginia.

Chaetomium gangligerum sp. nov.   Figs. 10–14.

Peritheciis fulvis, ostiolatis, magnitudinis mediae, ovatis in subglobosis gradientibus, 230–260 × 190–210 μ, sine cirrho, rhizoideis numerosis ad substratum affixis. Pilis lateralibus copiosis, gracilibus, septatis, apicibus acuminatis. Pilis terminalibus numerosis, distincte vel obscure septatis, tenuibus barbellatis, basi rectis vel arcuatis, fulvis, diametro 3.5–4.25 μ, apice spiraliter recurvatis. Ascis clavatis, octosporis, 50 × 18 μ, parte sporifera 36 μ. Ascosporis maturis brunneis, ovatis vel globoso-ovatis, umbonatis vel subapiculatis 12–18 × 7–11 μ. In media agar-agar cum liquore tuberis *Solani tuberosi,* et farina *Zeae Maydis,* nodulorum hypogaeorum fuscorum varietatem forma et magnitudine differentibus, copiosam producens.

Perithecia tawny yellow, ostiolate, moderately large, ovate to subglobose, 230–260 × 190–210 μ, without cirrhi, attached to the substratum with numerous rhizoids. Lateral hairs are numerous, slender, septate and gradually decreasing in diameter to the apex. Terminal hairs numerous, distinctly or obscurely septate, coated with many little barbules, straight or curved from the base, tawny yellow in color, 3.5–4.25 μ in diameter, spirally recurved at the apex. Asci clavate, eight-spored, 50 × 18 μ, spore part 36 μ. Mature ascospores brown, ovate to globose-ovate, umbonate to subapiculate, 12–18 × 7–11 μ. In agar-agar media enriched with potato and corn meal broth, dark colored bulbils are formed in a variety of shapes and in large numbers.

Type—isolated from wood samples which were under test in the Tropical Testing Chamber, Fort Belvoir, Virginia.

This species is easily distinguished in culture because of the dark-celled bulbils it produces in the agar.

Chaetomium velutinum sp. nov.   Figs. 15–18.

Peritheciis griseis, parvis, ovatis in subglobosis gradientibus, ostiolatis, 150–180 × 120–140 μ, interdum cum cirrho, rhizoideis numerosis, vix ad substratum affixis. Pilis lateralibus numerosis, gracilibus. Pilis termi-nalibus numerosis, septatis, gracilibus, barbellatis, diametro 4–5 μ, apice cum 1–3 convolutis.   Ascis longis, cylindricis, octosporis, 65 × 7 μ, parte sporifera 38–42 μ. Ascosporis maturis olivaceo-brunneis dilutis, limoniformibus, umbonatis vel subapiculatis, 6.75–8.5 × 4–6 μ.

Perithecia gray, small, ovate to subglobose, ostiolate, 150–180 × 120–140 μ, occasionally producing cirrhi, lightly affixed to the sub-stratum with numerous rhizoids.   Lateral hairs numerous, slender. Terminal hairs numerous, septate, graceful, 4–5 μ in diameter, cov-ered with little spines, and at the apex coiled in 1–3 convolutions. Asci long, cylindrical, eight-spored, 65 × 7 μ, spore part 38–42 μ. Mature ascospores dilute olive-brown, lemon-shaped, umbonate to subapiculate, 6.75–8.5 × 4–6 μ.

Type—isolated from a Japanese tent.   Culture was made by Dr. G. W. Martin and sent to the writer under the designation J-359-APO 565 (J-lm).

This small, silver-gray species is easily distinguished by its cylindrical asci in which the spores are monostichous.

Chaetomium atrobrunneum sp. nov.   Figs. 19–22.

Peritheciis fuscis, parvis, ostiolatis, globosis vel subglobosis, 80–120 × 80–110 μ, vel cirrhis vel sporis laxe acervatim in pilis terminalibus adhaerentibus, ad substratum rhizoideis stramineis affixis. Pilis lateralibus numerosis, tenuibus, distincte septatis, apice gradatim attenuatis. Pilis terminalibus longis, gracilibus, basi 3.75–4.75 μ diametro, saepe ramosis divaricatis, dis-tincte septatis, apice gradatim attenuatis. Ascis clavatis, octosporis 30 × 10 μ, parte sporifera 18 μ. Ascosporis maturis pallidis olivaceo-brunneis, longis et angustis, paullo fusiformibus, utrinque rotundatis vel subacutis, 10–12 × 5.5–7.5 μ.

Perithecia dark brown, small, ostiolate, globose to subglobose, 80–120 × 80–110 μ, with cirrhi or with masses of spores loosely held in the terminal hairs, affixed to the substratum with straw-colored rhizoids.   Lateral hairs numerous, slender, distinctly sep-tate, gradually narrowing in diameter to the tips.   Terminal hairs long, graceful, at the base 3.75–4.75 μ in diameter, often branched with wide angles to the main axis, distinctly septate, narrowing in diameter to a relatively sharp tip. Asci clavate, eight-spored, 30 × 10 μ, spore part 18 μ.   Mature ascospores dilute olive-brown, long

and narrow, somewhat fusiform, rounded to subacute on the ends, 10–12 × 5.5–7.5 μ.

Type—isolated from a molded mattress cover from Guadalcanal. Culture was made by Dr. G. W. Martin and sent to the writer under the designation J-1041-(3-J3).

This species is readily distinguished by the rich brown terminal hairs which are branched with wide angles.

Chaetomium seminudum sp. nov. Figs. 23–29.

Peritheciis parvis, nigris, vasiformis, 150 × 70 μ (100–165 × 65–85 μ), ostiolatis, cum cirrhis longis, ad substratum rhizoideis tenellis affixis. Pilis lateralibus et terminalibus uniformibus, paucis, septatis, basi 3.5–4 μ diametro, apice gradatim attenuatis. Ascis clavatis, octosporis, maturitatem ante dissolutis. Ascosporis maturis olivaceo-brunneis dilutis, globoso-ovatis, extremo altero rotundatis, extremo alio subacutis, 13.5 × 11 μ (9–14 × 7–8 μ), In media agar-agar cum liquore tuberis, *Solani tuberosi,* et farina, *Zeae Maydis,* chlamydosporas copiosas producens.

Perithecia small and black, vase-shaped, 150 × 70 μ (100–165 × 60–85 μ), ostiolate, with long cirrhi, attached to the substratum with translucent mycelium-like rhizoids. Lateral hairs and terminal hairs are alike, few in number, septate, at the base 3.5–4 μ in diameter, narrowing to a sharp tip. Asci clavate, eight-spored, liquefying before the spores mature. Mature spores light olive-brown, globose-ovate, the ends rotund to subacute in shape, 13.5 × 11 μ (9–14 × 7–8 μ). In agar-agar media enriched with potato and corn meal extract, myriads of chlamydospore-like bodies 10–15 μ in diameter are produced. Most of these bodies are borne on the ends of short slender stalks 30–50 μ in length by 2 μ; some are of intercalary origin (FIGS. 28 and 29). Scattered on the agar surface, very often, are hair-like structures which resemble the ornamental perithecial hairs (FIG. 27).

This interesting species was sent to the writer by Dr. J. C. Gilman, Iowa State College, Ames, Iowa. The plant is easily distinguished from all other described species of *Chaetomium* by its small seminude, vase-shaped perithecium and, in culture, by the copious formation of the chlamydospore-like bodies within the substratum.

Chaetomium cupreum sp. nov. Figs. 30–33.

Peritheciis parvis, ostiolatis, globosis vel ovatis, 110–120 × 120–130 μ, cirrhis conspicuis, rhizoideis tenellis ad substratum affixis. Pilis lateralibus

numerosis, gracilibus, distincte septatis, basi 3–3.5 μ diametro, apice 1–2 convolutis. Pilis terminalibus rigidis, distincte septatis, basi 4.5–6 μ diametro, apice 1–3 convolutis. Pilis lateralibus et terminalibus granulis cuprineis vestitis. Ascis clavatis, octosporis, $38 \times 13\,\mu$, parte sporifera $27\,\mu$. Ascosporis maturis, globoso-ovatis, subapiculatis, $10 \times 5.5\,\mu$ $(8.5–11.5 \times 5–5.5\,\mu)$.
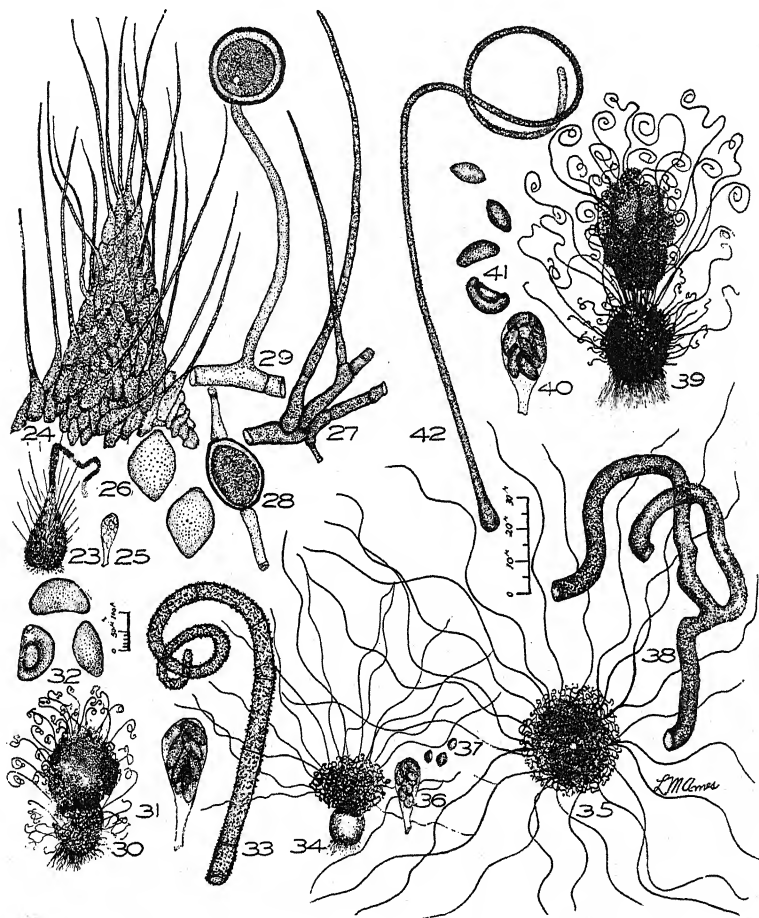
FIGS. 23–42. New species of *Chaetomium*.

Perithecia bright copper colored, small, ostiolate, globose to ovate, 110–120 × 120–130 μ, with conspicuous cirrhi; attached to the substratum with undifferentiated rhizoids. Lateral hairs numerous, slender, distinctly septate, 3–3.5 μ in diameter at the base, and at the apex with 1–2 convolutions. The terminal hairs are

rigid, distinctly septate, 4.5–6 μ in diameter at the base with 1–3 covolutions at the apex. Lateral and terminal hairs are covered with small grains which are copper colored. Asci clavate, eight-spored, $38 \times 13 \mu$, spore part 27μ. Mature ascospores globose-ovate, subapiculate, $10 \times 5.5 \mu$ ($8.5–11.5 \times 5–5.5 \mu$).

This bright colored species was first sent to the writer by Dr. Paul Marsh, U. S. Department of Agriculture, Beltsville, Md., who obtained it from deteriorating material collected in Panama Canal Zone. A second collection was sent to the writer by Dr. G. W. Martin who isolated it from material shipped from Guadalcanal. This species is distinguished from other Chaetomia by its bright copper colored hairs. The pigment granules dissolve in alcohol, ether, cellosolve, and xylol, but not in water.

Chaetomium causiaeformis sp. nov.   Figs. 34–38.

Peritheciis parvis, delicatulis, translucidis, ostiolatis, globosis vel sub-globosis, $80–100 \times 80–90 \mu$, sine cirrho, rhizoideis numerosis, vix ad substratum affixis. Pilis lateralibus paucis, gracilibus, translucidis, distincte septatis, tennibus, basi $1.25–2 \mu$ diametro. Pilis terminalibus typorum duorum: (a) aliis brevibus numerosis circa ostiolis, septatis, compositer ramosis, basi $3–4 \mu$ diametro, causia similibus. (b) aliis longis, undulatis, $4.25–5 \mu$ diametro, tenuibus barbellatis, apicibus acuminatis, usque ad 1800μ longis. Ascis clavatis, octosporis, $23 \times 8 \mu$, parte sporifera 18μ. Ascosporis maturis brunneis dilutis, ovatis vel subglobosis, $5 \times 4 \mu$ ($4.75–5.25 \times 3.0–4.5 \mu$).

Perithecia small, delicate, translucent, ostiolate, globose to sub-globose, $80–100 \times 80–90 \mu$, without cirrhi, rhizoids numerous, lightly attached to the substratum. Lateral hairs are few in number, slender, translucent, distinctly septate, tapering, at the base $1.25–2 \mu$ in diameter. Terminal hairs are of two types: (a) those which are short and arranged closely about the ostiole, septate and branched, at the base $3–4 \mu$ in diameter, in general simulating a hat. (b) those which are long, unbranched and undulating, $4.25–5 \mu$ in diameter, covered with very small barbules, tapering toward the apex, often reaching a length of 1800μ. Asci clavate, eight-spored, $23 \times 8 \mu$, spore part 18μ. Mature asci are dilute brown, ovate to subglobose, $5 \times 4 \mu$ ($4.75–5.25 \times 3.0–4.5 \mu$).

This species is distinguished by the most delicate perithecium among the Chaetomia that the writer has observed. This fungus was sent to the writer by Dr. G. W. Martin and was designated as J-1334.

Chaetomium succineum sp. nov.   Figs. 39–42.

Peritheciis magnitudinis mediae, globosis vel ovatis, 225–350 × 140–230 μ, ostiolatis, cirrhis frequenter provisis, rhizoideis tenellis ad substratum affixis. Pilis lateralibus numerosis, gracilibus, septatis. Pilis terminalibus numerosis, in cumulis autem laxiformibus, cirrhos fractos superportantibus. Pilis terminalibus gracilibus, diametro 3.5–4 μ basi, apicibus obtusis acuminatis, septatis, apice 1–3 convolutis laxis. Ascis clavatis, octosporis, 35 × 15 μ, parte sporifera 27 μ. Ascosporis maturis pallide olivaceo-brunneis, globoso-ovatis, utrinque rotundatis vel subacutis, 14 × 7.5 μ (12–15 × 7–8 μ).

Perithecia of medium size, globose to ovate, 225–350 × 140–230 μ, ostiolate, frequently provided with cirrhi, attached to the substratum with delicate rhizoids. Lateral hairs numerous, slender, septate. Terminal hairs numerous, of a beautiful amber color, formed in a loose cluster which holds fragmented cirrhi. Lateral hairs are graceful, 3.5–4 μ in diameter, septate, acuminate to a blunt apex which is coiled with 1–3 convolutions. Asci clavate, eight-spored, 35 × 15 μ, spore part 27 μ. Mature ascospores are of a pale olive-brown, globose-ovate, rounded to subacute at the ends, 14 × 7.5 μ (12–15 × 7–8 μ).

This species is distinguished by the loose cluster of slender amber-colored hairs which ornament the apex of the perithecium. The perithecia, in culture, are numerous but not crowded. Cultures were sent to the writer by Dr. G. W. Martin and by Mr. William B. Cooke, Pullman, Washington.

DISCUSSION

Type specimens of each species have been deposited at the Farlow Herbarium, Cambridge, Massachusetts, and co-types at the U. S. Department of Agriculture, Beltsville, Md.

Some of the species of *Chaetomium* described in this paper were isolated from rotting mattresses, tenting, knapsacks, clothing and other items of equipment from various islands in the Pacific combat areas. These plants were sent to the writer for identification and study by Dr. G. W. Martin and Miss Louise G. Isfort from the Jeffersonville Quartermaster Depot, Jeffersonville, Indiana. Several specimens were received from Mr. William B. Cooke, Pullman, Washington and Dr. Paul Marsh, Bureau of Plant Industry, Washington, D. C. For their kindness in sending the many specimens, and for the assistance of Dr. Hugh T. O'Neill at Catho-

lic University in revising my Latin descriptions, I wish to express my deep appreciation. Additional species, discovered by the writer, were found growing on material and equipment in the Tropical Testing Chamber, Fort Belvoir, Virginia. The first fungus belonging to the genus *Chaetomium,* as now understood, was described more than 131 years ago.

Kunze erected the genus *Chaetomium* in 1817 based upon the species *C. globosum;* a second species, *C. elatum,* was published by him a year later. In 1837 Corda emended Kunze's original description principally by describing the ostiolum; two new species, *C. indicum* and *C. murorum,* were added to the genus by him at that time. Descriptions of new species have appeared rather infrequently since the erection of the genus, and many of the later names have been determined subsequently to be synonyms or to have been erroneously applied. Three small monographic papers were published by Zopf (**1881**), Palliser (**1910**), and Bainier (**1910**). These were followed by a much more complete monographic treatment of the genera *Chaetomium* and *Ascotricha* in 1915 by Chivers. In this excellent monograph, of the 114 species and 14 varieties which were referred to the genus *Chaetomium,* he recognized only 28 species, 11 of which he described as new. Most of the species were beautifully illustrated, thus making identifications relatively easy and accurate. More recently, Tschudy (**1937**) described two species, *C. ochraceum* and *C. cancroideum,* which were isolated from decomposing reeds. In 1945 Hughes described a 4-spored species, *C. tetrasporum.* In the same year, Ames described three additional species, *C. dolichotrichum, C. microcephalum,* and *C. pachypodioides.* In a recent paper Skolko and Groves described two new species, *C. erectum* and *C. reflexum,* which were isolated from various types of seeds.

The species of *Chaetomium* described in this paper were grown in pure culture on the following medium:

NaNO$_3$	3.0 gms.
MgSO$_4$·7H$_2$O	0.5 gm.
K$_2$HPO$_4$	1.0 gm.
KCl	0.2 gm.
Potato extract	50 ml.
Corn meal extract	50 ml.
Distilled water to make	1,000 ml.

On this medium strips of sterilized paper or 5 oz. cotton cloth were placed as a source of carbon. The cultures were grown in an incubation chamber maintained at a temperature of 85° F. and at a relative humidity of 85%. The same features of the species, as herein described, should be obtained when grown on the medium and under the conditions outlined. Some growth characteristics were observed, during the present study, which were not previously associated with the genus.

Conidia have not been authentically described hitherto for *Chaetomium* in spite of the fact that two closely related genera, *Chaetomidium* and *Ascotricha,* produce them abundantly. Recently, the writer has observed bulbils produced, in the agar medium, by one species, *C. gangligerum,* see figure 14. These bodies have not been fully investigated as yet to determine their reproductive capacity. A second species, *C. seminudum,* produces abundant bodies, see figures 28 and 29, borne apically and singly on slender branches, or occasionally they are of intercalary origin. These bodies are produced submerged in the agar culture medium in such numbers that the agar appears cloudy. These chlamydospore-like bodies will be studied more critically and reported on subsequently.

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EXPLANATION OF FIGURES

FIGS. 1 and 2, mature perithecia of *C. turgidopilosum;* 3, detail of a terminal hair; 4 and 5, ascus and ascospores; 6, mature perithecium of *C. cristatum;* 7, ascus with contained ascospores; 8, detail of type (a) terminal hair; 9, mature ascospores; 10, mature perithecium of *C. gangligerum;* 11, ascus with contained ascospores; 12, detail of terminal hair; 13, mature ascospores; 14, detail of "knots" or bulbil development; 15, mature perithecium of *C. velutinum;* 16, ascus with contained ascospores; 17, detail of terminal hair; 18, mature ascospores; 19, mature perithecium of *C. atrobrunneum;* 20, detail of branched terminal hair; 21, mature ascospores; 22, ascus with contained ascospores; 23, mature perithecium of *C. seminudum;* 24, detail of perithecium apex; 25, immature ascus; 26, mature ascospores enlarged by oil mount and 15 × eye piece; 27, hairs from agar surface; 28 and 29, chlamydospore-like bodies enlarged by oil mount and 15 × eye piece; 30, mature perithecium of *C. cupreum;* 31, ascus with contained ascospores; 32, mature ascospores; 33, detail of terminal hair; 34 and 35, mature perithecia of *C. causiaeformis;* 36, ascus with contained ascospores; 37, mature ascospores; 38, detail of short terminal hair surrounding the ostiole; 39, mature perithecium of *C. succineum;* 40, ascus with contained ascospores; 41, mature ascospores; 42, detail of terminal hair.

WYNNEA AMERICANA

Richard P. Korf

(with 1 figure)

One of the rarer Operculate Discomycetes is *Wynnea americana* Thaxter, easily recognizable by the many spoon-shaped apothecia arising from a fleshy, hypogeous "sclerotium" (FIG. 1), the characteristically striate spores, and the eccentric ascus operculum. This short note is a report of some collections from New York and from West Virginia, extending the known range of this species.

In a recent and excellent paper, Le Gal (2) has discussed the morphology of the ascus tip in this and several other related fungi which comprise a new group, the Suboperculates, somewhat intermediate between Operculates and Inoperculates. Her treatment covers representatives of *Plectania* (sub *Sarcoscypha*), *Cookeina, Phillipsia, Pithya, Urnula, Rhizopodella* (sub *Urnula*), *Bulgaria* (sub *Sarcosoma*), *Pseudoplectania, Melascypha,* and *Wynnea.* In an earlier paper (3), delayed in publication, she had united these same genera into the family Sarcoscyphaceae, stressing spore ornamentation. Dr. Le Gal's disposition of these forms seems most natural, and the writer's observations have thus far been in full accord with hers.

Wynnea americana is known from several North American localities, including Tennessee, North Carolina, Ohio, and southern Pennsylvania (4). More recently it has been reported from additional Pennsylvania stations (1, 5).

Several unrecorded collections extend the northerly range of this species approximately sixty miles beyond the known limit at Meadville, Crawford County, Pennsylvania (4). Three collections made in the Lloyd-Cornell Preserve at Ringwood, New York, about seven miles east of Ithaca, are the most northerly collections known to the writer. Two collections from West Virginia, kindly communicated by Dr. H. L. Barnett, are also reported here.

Fig. 1. *Wynnea americana* Thaxter. Photograph, natural size, of a clump of apothecia; the soil surface was at the constriction just above the "sclerotium."

The specimens examined which extend the known distribution are deposited as follows:

NEW YORK—Gordon, Rea, et al., *Big Basin Forest, Allegany State Park, Cattaraugus Co., Aug. 16, 1935:* NYBG.—H. M. Fitzpatrick and C. T. Rogerson, *Ringwood, Tompkins Co., Sept. 26, 1947:* CTR; RPK.—R. E. Perkins and R. P. Korf, *same locality, Sept. 27, 1947:* CU–P 37137; NYBG; RPK.—do., *another collection:* RPK.

WEST VIRGINIA—H. L. Barnett, *Cooper Rock State Forest, Monongalia Co., August 30, 1947:* WVU.—W. C. Legg, *Mt. Lookout, Nicholas Co., November 1947:* WVU.

The writer wishes to express his appreciation to Mr. W. R. Fisher, who made the excellent photograph accompanying this note (CU–P 37137). In this specimen the hymenium was a light rose-pink color, much lighter than that usually ascribed to this species. The other two collections from Ringwood were well past their prime, and some deterioration had already occurred; in these the hymenial color was a deep purple-red. They more closely approximated the appearance of the photograph in the book by Dr. Seaver (**5**: plate 16), to whom the writer is indebted for the loan of material from the New York Botanical Garden.

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CYATHUS VERNICOSUS, ANOTHER TETRA-POLAR BIRD'S NEST FUNGUS

HAROLD J. BRODIE

(WITH 2 FIGURES)

The recent studies of Dr. Nils Fries of Sweden and of the writer concerning sexuality in the Nidulariaceae have shown that all species thus far examined are heterothallic and tetrapolar. The species of this group of fungi for which four-mating-type hetero-thallism has been established are:

Crucibulum vulgare Tul....................Fries, 1936 (3)
Cyathus stercoreus Schw. (De Toni)..........Brodie, 1948 (1, 2)
Cyathus striatus Willd......................Fries, 1936 (3)
Nidularia pisiformis (Roth.) Zell............Fries, 1948 (4)

To this list may now be added *Cyathus vernicosus* DC. This fungus is of fairly common occurrence in gardens, where it often grows among dead plant stems or around board edging. In green-houses it has been found on flats of soil. The species is readily recognized by its buff-grey color, wide flaring mouth and large peridioles (FIG. 2). The inside surface of the cup usually has a shiny or varnished appearance.

In 1920, Dr. Leva Walker (5) published an account of *C. vernicosus* mainly from the point of view of the developmental morphology of the fruit body. It was shown that basidiospores contain two nuclei and that two nuclei are regularly present in each cell of the diploid mycelium which bears clamp connections. Since Miss Walker did not succeed in obtaining a series of monosporous mycelia, the question of whether the fungus is homothallic or heterothallic was left unanswered.

By growing diploid mycelium on sterile loam, old leaves and half-rotten wood, Miss Walker obtained fruit bodies in the labora-tory and full details of every step in the development of the fruit bodies and their peridioles were described.

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MATERIALS AND METHODS

The first attempt made by the writer to culture this fungus was in 1941. An abundant supply of fruit bodies was found in a garden in Winnipeg, Canada, where the little cups were growing around the bases of dead chrysanthemum stalks. Spores from chopped peridioles were placed in distilled water and in various nutrient solutions at room temperature. So few spores (1–3%) germinated in any test that the attempt to obtain a series of monosporous mycelia was abandoned. Several efforts were made subsequently to germinate enough spores for study, but always with the same unsatisfactory result. Similar difficulty was reported by Miss Walker (5).

When it had been learned that the basidiospores of *Cyathus stercoreus* · germinate well after being subjected to a temperature of 40° C. for 48 hrs.—see Brodie, 1948 (1)—fresh material of *C. vernicosus* was sought. The writer is indebted to Mr. Garnet Best of Winnipeg, Canada, for three fruit bodies collected by him in that city, Oct. 19, 1947. These specimens provided the single-spore cultures used in the present investigation.

The spores were not tested for germinability until Nov. 19, 1948, exactly a year after the fruit bodies had been collected. Peridioles were cut open under aseptic conditions and spores in a distilled water suspension were incubated at 40° C. for 48 hours, using exactly the same technique as has been described by the writer (1) in his report on *C. stercoreus*.

Plate dilutions were made and single spores cut out under the microscope. The mycelia that developed were transferred to tube slants of a special medium compounded as follows: Bacto agar, 20 gm.; maltose, 5 gm.; dextrose, 2 gm.; glycerine, 2 gm.; peptone, 0.2 gm.; asparagine, 0.2 gm.; yeast extract, 2 gm.; magnesium sulphate, 0.5 gm.; calcium nitrate, 0.5 gm.; dihydrogen potassium phosphate, 0.5 gm.; ferrous sulphate, trace; distilled water to make 1 liter.[1] Using this medium, optimum vegetative growth at room

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[1] Two of the ingredients were inadvertently omitted from the formula as given in a previous article (2) and the corrected formula is therefore given herewith.

temperature was obtained when the acidity of the medium was adjusted to pH 6.5.

Sixty monospore mycelia were isolated but only twenty-nine of these were used in the study of pairing reactions.

SPORE GERMINATION AND HAPLOID MYCELIA

The basidiospores of *C. vernicosus* are moderately thin-walled, colorless and (in the collection referred to above) measure mostly $7 \times 11\,\mu$. Spores began to germinate 24 hours after the heat treatment, but some germinated tardily a day later. From 40–60% of the spores germinated in each of the different spore samples. A single stout germ tube developed from each spore and this rapidly grew into a haploid mycelium.

Haploid mycelia growing on agar plates are fluffy and fine-textured. Of twenty-nine, selected at random for pairing, only six showed any tendency to produce the coarse mycelial cords characteristic of the diploid mycelia, the rest remained fine-textured throughout numerous transfers.

Haploid mycelia show only slight morphological differences when monospore cultures are compared. As to color, they are all snow-white when freshly transferred and most of them become a dull ivory color about two weeks later. Four of the series of the original sixty isolates were buffy brown (Ridgway). No attempt has been made to study the inheritance of color because the color differences between haploids are not so striking as they are in *C. stercoreus,* nor are the colors so constant from transfer to transfer.

No haploid mycelium has been observed to produce oidia nor to produce fruit bodies in culture.

PAIRING REACTIONS AND DIPLOID MYCELIUM

Twenty-nine haploid mycelia were paired in all possible combinations in the usual way on agar slants. Diploid mycelium began to develop between certain pairs ten days later. All mycelial pairs were examined for clamp connections at the end of two weeks. On the basis of their mating reactions, the haploid mycelia fell into four mating types with the following distribution:

Mating type   Culture number[2]

AB...........1, 4, 5, 7, 15, 16, 20, 29
ab............2, 8, 14, 24, 27
Ab...........3, 6, 10, 13, 17, 19, 21, 22, 23, 25, 26, 28, 30
aB...........12, 18

It would thus appear that *Cyathus vernicosus,* like all other members of the Nidulariaceae examined up to the present time, is heterothallic and tetrapolar. The distribution of haploid mycelia among the four sex groups is very unequal, similar to the distribution previously noticed by the writer (1) in *C. stercoreus.* From a small number of pairings, this might be considered to have no special significance. However, Dr. Nils Fries has informed the writer (by letter) that he attempted an analysis of sexuality of the European *C. olla* (Batsch) Pers. (*C. vernicosus*) and found that 23 monosporous mycelia fell into four groups in the ratio of $1:11:10:1$. Because two groups were represented by only one mycelium each, Fries did not publish his results. Fries' finding furnishes corroboration for the present writer's opinion that this unequal distribution is a common occurrence and that there is some factor operative in determining the unequal distribution of mycelia as to mating type. This may well be similar to what has been observed in *C. stercoreus* (1).

Diploid mycelia bearing clamp connections are mostly white or pale ivory in color and are composed of hyphae that are somewhat coarser than are those of haploid mycelia. The hyphae of the diploid mycelium tend to become aggregated into loose ropes or cords, which give to a colony on an agar plate a characteristic radiate appearance. Some coarse mycelial cords form as colonies get older, and on these the fruit body rudiments develop. Cords of this kind are not as abundant on the diploid mycelium of *C. vernicosus* as they are on *C. stercoreus* mycelium.

PRODUCTION OF FRUIT BODIES IN CULTURE

Whereas the addition of filter paper as a source of cellulose to the special medium referred to above induced abundant normal fruiting of *C. stercoreus* (1, 2), this procedure failed to cause the

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[2] Culture No. 9 was eliminated from the series when it failed to continue growth after two transfers.
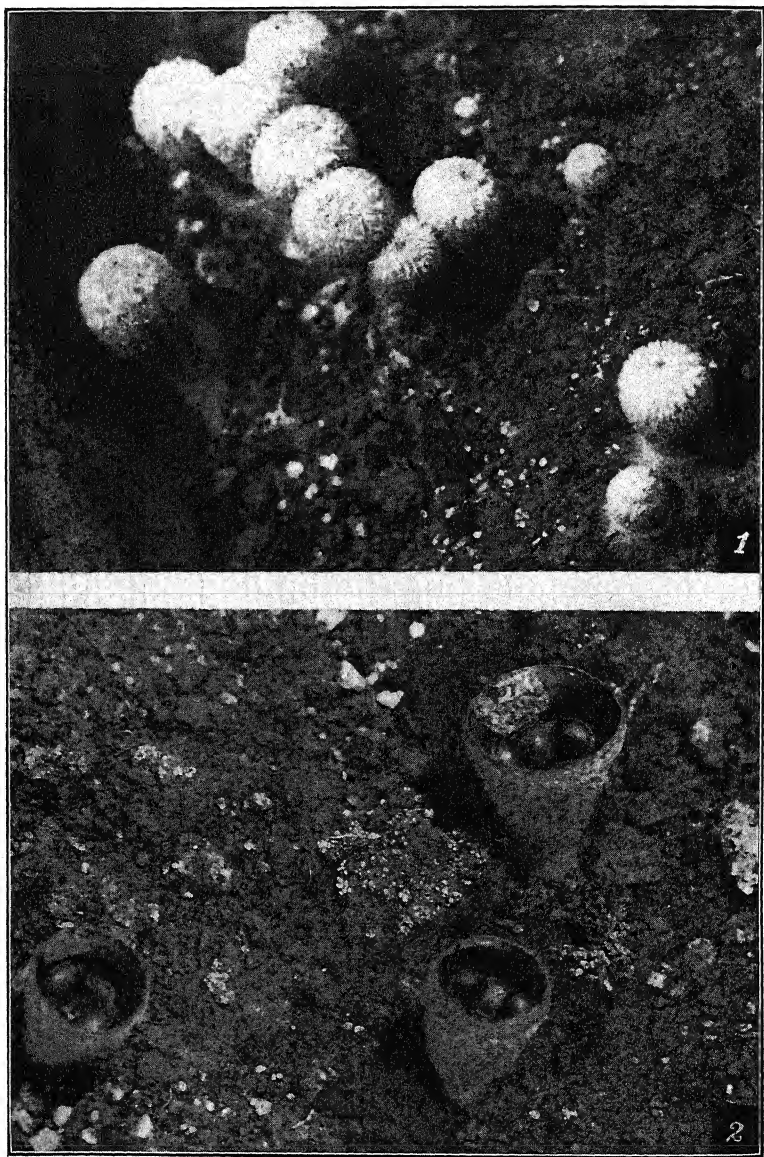
Figs. 1–2. *Cyathus vernicosus.*

mycelia of *C. vernicosus* to fruit.   Even the development of knots on the mycelium that indicates the beginning of fruit body formation took place only on three cultures that were six weeks old and none of the knots continued to grow.

Cultures were then transferred to sterilized mixtures of old leaves, rotting wood, etc., following Miss Walker's (5) suggestion, but in no instance did fruit bodies appear on any culture although some of these were kept for three months.

Finally it was decided to resort to the use of soil.   Mycelium (and agar medium) from five slants in flasks, each culture a month old, was placed in a clean 8 in. flower pot.   The culture material was then covered to a depth of one inch with a sifted sterilized loam mixture and the soil was pressed down gently.

One week later, mycelial strands appeared on the surface of the soil and a few fruit body rudiments were visible.   By the end of another week, seventy fruit bodies had developed on the culture, all of which opened normally in a few days (FIGS. 1, 2).

Regarding this rapid development of fruit bodies after the addition of soil, it seems unlikely that the soil could supply any nutritional deficiency which could induce fruiting so soon after application.   It appears more probable that aeration (texture of the medium, etc.) was not properly provided by the agar slants but was provided by the loose covering of soil.   To date, several other cultures of *C. vernicosus* have been induced to fruit in this way, but other methods have proven unsuccessful.

Although several diploid mycelia, each representing combinations of different haploid mycelia, have been fruited, there is little variation in the morphology of the fruit bodies: nothing comparable to the extreme variability characteristic of *C. stercoreus* fruit bodies has been observed.

Most detail concerning the morphology of the fruit bodies is being reserved for future publication.   However, because the photographs illustrate two noteworthy features of this species, attention may be drawn briefly to these.   It is not generally recognized that fruit bodies of *C. vernicosus* develop a basal mass of hyphae (the so-called "emplacement") comparable with the large and conspicuous ball found at the base of the fruit body of such species as *C. striatus* and *C. stercoreus*.   In *C. vernicosus* this

emplacement develops early and may be seen as a mycelial mat around the base of a young fruit body (FIG. 1). As development proceeds, soil becomes incorporated into the emplacement so that its size and full extent are appreciated only when soil is lifted from below the base of the cup.

The epiphragm or covering over the mouth of the unopened fruit body usually ruptures by an irregular tear across the diameter in *C. striatus* and *C. stercoreus*. The epiphragm of all specimens of *C. vernicosus* developed in the writer's cultures seemed to rupture in a circumscissile manner, the epiphragm withdrawing as a shrunken disk to one side of the peridium (FIG. 2).

SUMMARY

1. Basidiospores of *Cyathus vernicosus* were germinated one year after collection by subjecting them to a temperature of 40° C. for 48 hrs. in distilled water suspension.

2. Sixty single-spore mycelia were cultured, of which 29 were used in a study of heterothallism.

3. Single-spore mycelia are haploid. The mycelia are quite uniformly white and fluffy, an occasional one showing grey-brown coloration.

4. When paired, haploid mycelia fall into four mating-type groups with uneven distribution of mycelia in the groups. The fungus is heterothallic and tetrapolar.

5. Diploid hyphae bear clamp connections and tend to become aggregated into cords upon which fruit body rudiments arise.

6. Diploid mycelia were induced to fruit only by covering cultures on agar slants with an inch of sterile soil.

7. Each fruit body of *C. vernicosus* has an "emplacement" or mass of basal hyphae which becomes compacted into a solid ball within the soil. This structure is similar to the emplacements of *C. striatus* and *C. stercoreus*.

8. The epiphragm of *C. vernicosus* ruptures most frequently in a circumscissile manner and withdraws to one side of the cup as a shrunken disk.

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DESCRIPTIONS OF ILLUSTRATIONS

FIG. 1. Young unopened fruit bodies of *Cyathus vernicosus* developed on an agar culture which had been covered two weeks before by one inch of sterile soil. Two young specimens in the lower right show the early development of the basal emplacement hyphae. × 2.

FIG. 2. Fruit bodies of *Cyathus vernicosus* a few hours after they had opened. The epiphragm in the largest specimen had withdrawn to the side of the mouth where it remained as a shrunken disk. As specimens get older, the mouth tends to flare outward more than in these young specimens. × 2.

STUDIES IN THE GENUS OTIDEA *

BESSIE B. KANOUSE

(WITH 21 FIGURES)

During the collecting season of 1948, from late June to October, the University of Michigan maintained a botanical expedition in Mt. Rainier National Park, Washington. Dr. A. H. Smith who conducted the survey was assisted by Henry A. Imshaug and Emory G. Simmons. Dr. D. E. Stuntz of the Department of Botany, University of Washington, joined the group in July. They obtained abundant collections of *Otidea* species which, with the collections in the University of Michigan Herbarium, form the basis of this investigation. The writer recognizes ten species and three varieties. This number materially exceeds the number in previous published reports for North America. Seaver (1928) reported three under the name *Scodellina*. One new species, *O. rainierensis*, and one new variety, *O. alutacea* var. *microspora*, are described from the Mt. Rainier collections and one new species, *O. Kauffmanii*, is named from Michigan material. The types of new species described here are deposited in the University Herbarium of the University of Michigan.

The genus *Otidea* was established by Fuckel (1869–1870) for *Peziza leporina* Pers. ex Fr., *P. onotica* Fr., *P. cochleata* L. and *P. abietina* Pers. The principal diagnostic character by which species of *Otidea* differ from those in *Peziza* is the presence of a split in the apothecium. Fuckel (l.c.) in his diagnosis of the genus described the paraphyses as "filiform subclavate" although he included *O. leporina* and *O. onotica* in which the paraphyses are hooked and not subclavate. Since the genus was established, several species which have filiform or filiform-subclavate paraphyses have been described for it. Boudier (1885) considered the straight paraphyses as distinctive and established the genus *Wynnella* based in part on such paraphyses. *O. auricula* Schaeff. was

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* Papers from the Herbarium of the University of Michigan.

made the type of *Wynnella*. Rehm (1887–1896) reduced this genus to subgeneric rank. The writer does not regard *Wynnella* as sufficiently different from *Otidea* to consider it as a genus.

The paraphyses in the two new species described in this paper exhibit a third type for the genus. In both of these they are filiform and the apices are expanded into broadly clavate, pyriform or globose to subglobose heads. No previously described species of *Otidea* with this type of paraphysis is known to the writer. *O. fibrillosa* (Currey) Mass. was so described, but the fungus is not an *Otidea*. The spores are described as eguttulate, and the exterior of the apothecia as tomentose. As illustrated by Cooke (1879) the apothecia are not split. Our species are obviously not to be considered *O. fibrillosa*. Besides the split apothecia other characters that *Otidea* species have in common are: elliptical biguttulate (rarely uniguttulate) spores, absence of blue coloration in iodine solution, and the prosenchymatic structure of the hypothecium. The excipular layer shows variation but no more than might be expected as differences at the species level. In general this layer is composed of hyphae which are divided into cells so as to give a pseudoparenchymatic appearance. The cells are subglobose, hexagonal to long cylindrical, and exhibit many irregularities of shape and size. This layer may be relatively thick —up to $300\,\mu$, or as narrow as $50\,\mu$. It is yellowish to brownish yellow in color. The transition from the hypothecial layer is usually gradual but it may be sharply differentiated, as it is in *O. auricula*. The outermost layer in some species is fairly even, as in *O. Cantharella* v. *minor* Boud., but in most species the hyphal ends are gathered up into small pyramidal aggregations frequently with short protruding chains of from three to six cells. Such chains are found in connection with the aggregations of hyphae or they may arise from a surface that does not produce the conspicuous bunches of cells. The surface cells are not sufficiently developed to produce more than a very slight tomentosity on the cup proper. The basal portion of the cup and the stipe may be definitely tomentose. In *O. auricula* as represented in the North American collection cited in this paper, the outermost layer is a definite palisade layer of elongate cells rounded at their apices (FIG. 7). Such a distinct palisade layer has not been found

in other species studied by the author nor has reference to it been seen in the literature except in Massee's description of *O. neglecta*. The reader is referred to the remarks concerning this situation in the discussion following *O. auricula*. In *O. grandis* and *O. Smithii* there exists a condition that is peculiar to these species. The excipular layer is covered with minute, subglobose granules golden brown in color.   They give the excipular cells a scurfy appearance and emphasize the brown color, and, in *O. grandis,* the mealy texture of the outside of the apothecia.   In a water mount they become detached easily.   Boudier (**1905–1910**) illustrated them for *O. umbrina,* but referred to them only in the description of the plate.   They were found in all of the collections of *O. Smithii* and *O. grandis* examined by the author.

OTIDEA (Fr.) Fuckel

Apothecia sessile to stipitate, gregarious, often cespitose, occasionally fused at their bases, size varying, from 1 cm. to 10 cm. in height, elongate to ear-shaped or truncate, split to the base on one side, glabrous to furfuraceous, hypothecium prosenchymatic, exciple pseudoparenchymatic; asci cylindrical, 8-spored; spores usually smooth, biguttulate (infrequently uniguttulate); paraphyses filiform and frequently branched below, apices straight, bent, hooked, clavate, or globose; no blue color reaction in iodine.

Type of the genus: *Otidea leporina* (Fr.) Fuckel.

KEY TO SPECIES

1. Paraphyses hooked or bent at their apices..........................2
1. Paraphyses not hooked or bent at their apices.......................8
2. Apothecia typically ear- or spoon-shaped...........................3
2. Apothecia truncate, not ear-shaped.................................6
3. Apothecia large, deep vinaceous brown, concolorous.........*O. Smithii*
3. Apothecia some shade of yellow or yellow-brown....................4
4. Apothecia bright yellow with rosy tints to the hymenium; spores 12–
     14 × 6–7 (8) μ...*O. onotica*
4. Apothecia lacking rosy tints in hymenium..........................5
5. Apothecia medium to large, clear yellow; spores 10–12 (13) × 5–6 μ....
                                                          O. concinna
5. Apothecia medium sized, dull yellowish brown; spores 12–14 × 6–8 μ....
                                                    O leporina v. *typica*
5. As above but spores 8–11 × 5–6 μ.................*O. leporina* v. *minor*

6. Apothecia usually densely cespitose, medium to large, alutaceous outside, wood-brown inside; spores 14–16 × 7–9 μ..........*O. alutacea* v. *typica*

6. As above but spores 9–11 × 5.5–6.5 μ..........*O. alutacea* v. *microspora*

6. Apothecia not alutaceous in color.................................7

7. Apothecia small to medium, exterior dark brown, mealy; spores 14–17 × 6–7 μ..*O. grandis*

7. Apothecia small, dull brownish yellow; spores 10–11 (12) × 6–7 μ.....
O. Cantharella v. *minor*

8. Paraphyses not enlarged at apices; apothecia large, ear-shaped, red-brown, mahogany outside, nearly concolorous within; spores 23–25 × 12–16 μ...
O. auricula

8. Paraphyses with apices enlarged.....................................9

9. Paraphyses with broadly clavate to subglobose heads................10

9. Paraphyses with numerous notched branchlets near apices..*O. abietina*

10. Apothecia large, wood-brown outside, drab gray inside................
O rainierensis sp. nov.

10. Apothecia small, yellowish tan to yellowish buff, concolorous..........
O. Kauffmanii sp. nov.

OTIDEA LEPORINA (Fr.) Fuck. var. TYPICA (FIGS. 1, 2).

Apothecia gregarious or cespitose, usually elongate–ear-shaped, split to the base on the short side, 1–4 cm. in height, 1–3 cm. in width, narrowed below into a stipe variable in length up to 6 mm. long, outside "hazel" (R.)* to "cinnamon rufus" (dry), inside "wood brown," "avellaneous" to "Sayal brown" (dry), stipe creamy white; hypothecium composed of hyaline hyphae interwoven; excipular layer thin (50–75 μ), composed of irregularly arranged subglobose to polygonal cells yellowish in color, the outermost layer giving rise to short chains and aggregations of cells; asci cylindrical, 140–170 × 10–12 μ, 8-spored, not turning blue in iodine; spores elliptical, smooth, slightly colored yellowish, 12–14 × 6–8 μ, biguttulate; paraphyses filiform, hyaline, apices slightly thickened, hooked.

HABITAT: On ground.

DISTRIBUTION: Colorado, Maryland, New York, Oregon, Washington.

MATERIAL EXAMINED: Rehm, Ascomyceten *1627a, 1627b;* C. A. Brown, Lake Quinault, Wash., Oct. 11 and Oct. 13, 1925; F. B. Cotner, Tolland, Colo., Aug. 27 and Aug. 28, 1920; J. B. Flett, Bremerton, Wash., Oct. 26, 1942; W. Haydon, Marshfield, Ore., Oct. 12, 1914; C. H. Kauffman and E. B. Mains, Lake Placid, New York, Sept. 10, 1914; C. H. Kauffman, Lake Cushman, Wash., Oct. 2, 1915; Cabin John, Md., Aug. 22, 1919;

*Ridgway, R., 1912. Color Standards and Color Nomenclature, Washington, D. C.

Tolland, Colo., Aug. 27, 1920; Lake Quinault, Wash., Oct. 11 and Oct. 13, 1925; Takilma, Ore., Dec. 10, 1925; E. G. Simmons, Longmire, Wash., Sept. 4, 1948, *2172;* A. H. Smith, Lake Crescent, Wash., Oct. 28, 1935, *3380;* Lake Tahkenitch, Ore., Nov. 21, 1935, *3585;* Lower Nisqually River, Wash., Sept. 2, 1948, *30888,* Nancy Jane Smith, Longmire, Wash., Aug. 26, 1948 (A. H. Smith *30664*); L. E. Wehmeyer, Mt. Hood, Ore., Oct. 15, 1922.
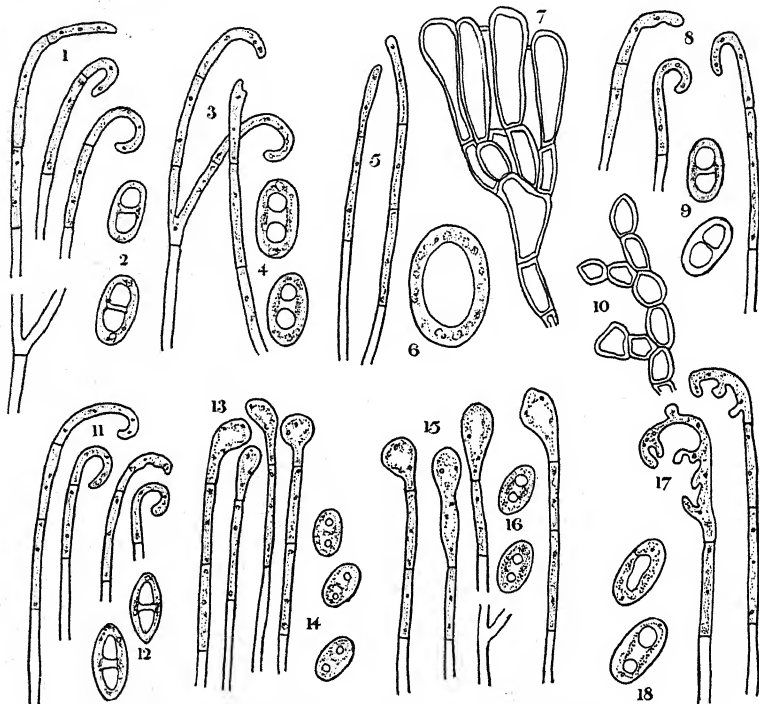
FIGS. 1-18. Microscopic characters in *Otidea*.

OTIDEA LEPORINA (Fr.) Fuck. var. MINOR (Rehm) Sacc. Syll. Fung. **8**: 94. 1889.

Like var. *typica* except that the asci and spores are smaller. The spores measure 8–11 × 5–6 μ.

HABITAT: On ground.

DISTRIBUTION: California, Michigan, New York, Oregon, Washington.

MATERIAL EXAMINED: C. H. Kauffman, Takilma, Ore., Nov. 29, and Dec. 10, 1925; C. H. Kauffman and Ethel Taylor, Marquette, Mich., Aug. 27, 1909; C. H. Kauffman and E. B. Mains, Chelsea, Mich., Aug. 2, 1915;

C. H. Kauffman and C. A. Brown, Takilma, Ore., Dec. 7 and Dec. 10, 1925; E. B. Mains, Rock River, Mich., Aug. 20, 1932, *32–177;* Baker Lake, Wash., Aug. 31, 1941, *6180;* A. H. Smith, Warrensburg, N. Y., Sept. 14, 1934, *1002;* Crescent Beach, Wash., Sept. 24, 1935, *2594;* Trinidad, Calif., Nov. 30, 1935, *3690;* Whitmore Lake, Mich., Sept. 26, 1936, *4929;* George Reserve, Pinckney, Mich., July 7, 1937, *6454;* Lower Tahoma Creek, Wash., Aug. 20, 1948, *30372;* L. E. Wehmeyer, Mt. Hood, Ore., Sept. 28, and Oct. 1922.

OTIDEA ONOTICA (Fr.) Fuck. Symb. Myc. 329. 1869–70. (FIGS. 10, 19).

Apothecia gregarious, often cespitose, substipitate to stipitate, elongate, typically spoon- or ear-shaped, margin involute, divided to the base on one side, arising from a mass of debris held together with mycelium, base white tomentose, 6–10 cm. in height, 5–6 cm. in width (wider if expanded), externally "ochraceous
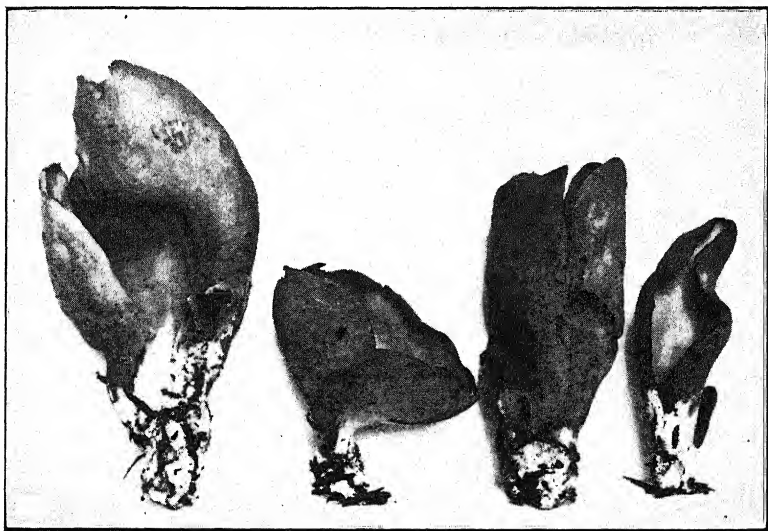
Photo A. H. Smith.

FIG. 19. *Otidea onotica.*   × ½.

orange," "cinnamon," "orange buff" (dry), interior "pinkish cinnamon," "ochraceous buff" to "apricot buff" with tints of rose; hypothecium composed of hyaline hyphae densely interwoven; excipular layer 50μ in thickness, composed of large cell-like hyphae from the outermost layer of which arise chains and irregular groups of cells; asci cylindrical, $160–200 \times 9–11 \mu$, not turning blue in iodine; spores elliptical, obliquely uniseriate, hyaline or colored faintly yellowish, smooth, biguttulate, 12–14 ×

6–7 (8) μ; paraphyses filiform, usually strongly hooked, frequently forked below.

Habitat: On the ground, usually in conifer forests.

Distribution: California, Michigan, New Hampshire, Oregon, Washington.

Discussion: The apothecia are larger and more brightly colored than in *O. leporina*. Since the time of Fries mention has been made of the rosy color present in the inside of the cups. It is strongly indicated even in dry specimens. The depth of color frequently approaches "zinc-orange." *O. leporina,* with which it has been confused, is duller in color and seldom approaches the size of *O. onotica.*

Material examined: H. A. Imshaug, Nisqually River, Wash., Aug. 30, 1948, *2116;* C. H. Kauffman and C. A. Brown, Takilma, Ore., Dec. 2, 1925; E. B. Mains, Randolph, N. H., Aug. 19, 1938, *4205;* Sept. 5, 1937, *4180;* E. G. Simmons, Lower Tahoma, Wash., Sept. 5, 1948, *2178;* A. H. Smith, LakeTahkenitch, Ore., Nov. 10, 1935, *3414;* Belknap Springs, Ore., Oct. 23, 1937, *8127;* Milford, Mich., July 2, 1940, *15166;* Chelsea, Mich., July 31, 1937, *6719;* Oregon Caves, Ore., Dec. 1, 1937, *9322;* Lower Tahoma Creek, Wash., Aug. *22, 30441,* Aug. 25, *30579,* Aug. 27, *30674,* Sept. 8, 1948, *30990,* Sept. 20, 1948, *31544;* Helen Smith, California Line, Nov. 20, 1937; A. H. Smith, Smith River, Calif., Oct. 16, 1937, *8940;* L. E. Wehmeyer, Oct. 13, 1922.

Otidea concinna (Pers. ex Fr.) Sacc. Syll. 8: 96. 1889.

Apothecia solitary to cespitose, truncate, strongly folded into convolutions, arising from a stout stem-like base, 2–3 cm. high, 3–4 cm. wide, color "pinkish cinnamon" to "Sayal brown" (dry) with a suggestion of yellow (said to be clear yellow when fresh), concolorous, whitish tomentose below; hypothecium composed of coarse, loosely woven hyaline hyphae; excipular layer narrow, pseudoparenchymatic, outer surface of large cell-like segments irregularly arranged, chains of cells present; asci cylindrical, 125–175 × 8–10 μ, 8-spored, not turning blue in iodine; spores elliptical, 10–12 (13) × 5–6 μ slightly colored yellowish, smooth, biguttulate; paraphyses filiform, sometimes forked below, apices hooked or bent.

Habitat: On the ground.

Distribution: Idaho, Washington, Sweden.

Discussion: This species is represented for North America in the University of Michigan Herbarium by three collections. These

collections and that of Rehm: Ascomyceten No. 1628 are indistinguishable in the dry condition as well as in their microscopical characters. The cups are much folded. One cup in the Idaho collection was measured after being soaked in water and the ruffled edge measured 20 cm. in circumference. Letellier (1829–1842) has an excellent illustration of this fungus.

Material examined: Rehm: Ascomyceten *1628* Sweden. Mycobiota of North America, Wm. B. Cooke, Latah Co., Idaho, *285* (distributed as *O. leporina*); H. A. Imshaug, Nisqually River, Wash., Aug. 30, 1948, *2127;* E. G. Simmons, Fish Creek, Mt. Rainier Nat. Park, Wash., Aug. 25, 1948, *2067.*

Otidea Cantharella var. minor Boud. Icon. Myc. 4, p. 181. 1905–1910. Vol. 2, pl. 326.

Apothecia solitary to gregarious, small, 1–1.5 cm. in height, 1–2.5 cm. broad, truncate, split on the short side to the base, edges of split enrolled, pale yellow outside, concolorous, drying "ochraceous buff" to "light ochraceous buff," substipitate, creamy white below (dry); hypothecium composed of hyaline hyphae densely interwoven; excipulum pseudoparenchymatic, cell-like hyphae thick-walled, yellowish, forming a fairly regular outer layer, not arranged in aggregations, palisades or chains; asci cylindrical, 140–160 × 10–12 μ, 8-spored, not turning blue in iodine; spores elliptical, smooth, biguttulate, 10–11 (12) × 6–7 μ, obliquely arranged in the asci; paraphyses filiform, hyaline, septate, branched below, apices bent or hooked, slightly thickened above and reaching 3 μ in width.

Habitat: On the ground.

Distribution: Colorado, Washington.

Material examined: E. B. Mains, Wild Basin, Rocky Mountain National Park, Colo., Sept. 1, 1940, *5270;* A. H. Smith, Lower Tahoma Creek, Wash., Sept. 8, 1948, *31033.*

Otidea alutacea (Fr.) Bres. var. typica (Figs. 3–4, 20).

Apothecia cespitose, frequently in large dense clusters, irregularly contorted, bases sometimes joined, infrequently solitary, truncate, sub-sessile or short stipitate, 2–6 cm. in height, 2–4 cm. in width, smooth, glabrous, drying wrinkled, exterior "tawny olive," "pinkish buff" or "cinnamon buff" to "clay color," "wood brown" (dry); hymenium "avellaneous" to "wood brown" (fresh), fragile; hypothecium composed of hyaline hyphae densely interwoven;

excipular layer pseudoparenchymatic, up to $200\,\mu$ thick, cell-like unit subglobose, yellowish, ending in a narrow marginal layer of irregular chains and loosely arranged groups of hyphal segments; asci cylindrical, 150–200 (250) × 8–10 μ, not turning blue in iodine; spores narrowly elliptical, smooth, 14–16 × 7–9 μ, biguttulate, slightly colored yellowish, uniseriate or obliquely arranged in the asci; paraphyses filiform, occasionally branched below one or two times, septate, hyaline, apices hooked.

HABITAT: On ground in coniferous forests.

DISTRIBUTION: California, Washington.

MATERIAL EXAMINED: Mycobiota of North America, Wm. Bridge Cooke *284* (distributed as *Otidea grandis*); H. A. Imshaug, Lower Tahoma Creek, Wash., *759, 830, 849, 859, 965, 1124, 1125, 1225*; E. G. Simmons, Longmire, Wash., July 28, 1948, *1722*, Aug. 30, 1948, *2106, 2108;* A. H. Smith, Olympic Nat. Park, Wash., Sept. 27, 1941, *17338* (reported by the writer (1947) provisionally as *O. felina.*); Lower Tahoma Creek, Wash., July 31 to Aug. 23, 1948, *29284, 29295, 29299, 29302, 29411, 29416, 29418, 29507, 29538, 29547, 29595, 29615, 29672, 29714, 29840, 29937, 30099, 30194, 30275, 30444, 30555, 31008, 31168;* Paul Rea, Santa Barbara Co., Calif., Dec. 31, 1941, *1081*.

Otidea alutacea var. microspora var. nov.

Apothecia solitaria aut caespitosa 5–8 cm. alta, truncata, pallide hyalino-flava, subtus albida, sicco pallide lutea usque roseo-lutea; sporis 9–10 ×
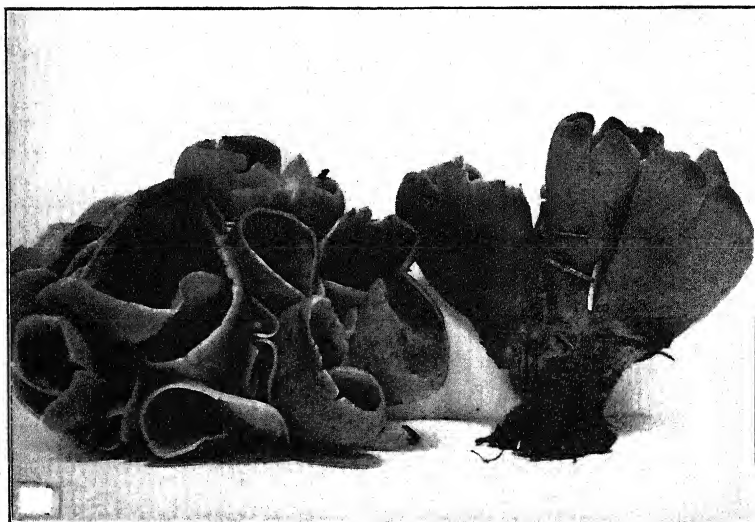
Photo A. H. Smith.

FIG. 20. *Otidea alutacea* var. *typica.* × ½.

5.5–6.5 (7) μ; hypothecio, excipulo, et paraphysibus varietatis typicae similibus.

Ad humum, Crescent City, Calif., 3 Dec. 1937. A. H. Smith. No. *9351*, Typus.

Apothecia solitary or cespitose, 5–8 cm. in height, truncate, pale clear yellow (fresh), whitish below, drying "pale buff" to "pinkish buff"; asci 175–200 × 7–10 μ, frequently with long rooting base, spores 9–10 × 5.5–6.5 μ; hypothecium thick, up to 350 μ; exciple thin; paraphyses with narrowly clavate apices, straight or hooked.

HABITAT: On ground.

DISTRIBUTION: California, Washington.

DISCUSSION: The differences between var. *typica* and var. *microspora* lie in the spore size and in the color of the apothecia. In var. *microspora* the color is paler and more yellow, and the spores are smaller.

MATERIAL EXAMINED: Olympic Hot Springs, Olympic Nat. Park, Wash., Oct. 8, 1941, A. H. Smith *17699*, **type**; reported by the writer (**1947**) provisionally as *O. felina* (Pers. ex Fr.) sensu Bres. A. H. Smith, Crescent City, Calif., Dec. 3, 1937, *9351*; Lower Tahoma Creek, Wash., Aug. 23, 1948, *30502*.

OTIDEA AURICULA (Cke.) Mass. Grev. **21**: 65. 1894 (FIGS. 5, 6, 7, 21).

Apothecia gregarious or solitary, narrowly elongate, ear-shaped, split on one side to the base, edges enrolled, stem-like base short, usually grooved, becoming horny when dry, 3–7 cm. in height, 1–2 cm. in width (3–4 cm. if flattened out), smooth, bay to chestnut brown shading to "clay color" at the base, margin sometimes "Sayal brown," inside darker, when dry dull purplish brown inside and outside; hypothecium composed of hyaline hyphae densely interwoven; excipular layer composed of pseudoparenchymatic hyphae, 100 μ in thickness, the outermost hyphal segments arranged in a definite palisade layer, elongated, ends rounded at the apices, containing brown coloring matter, color soluble in water; asci cylindrical, 300–400 × 15–18 μ, 8-spored, not turning blue in iodine; spores hyaline, smooth, containing one large central oil drop, 22–25 × 12–16 μ, uniseriate; paraphyses filiform, becoming clavate at the apices, 6 μ in diameter, containing brown coloring matter.

HABITAT: On the ground.

DISTRIBUTION: Michigan, Montana.

DISCUSSION: This species is not to be confused with a similar fungus which is white outside instead of brown. Massee has named the fungus with the white exterior *O. neglecta* Massee. The uniform yellowish brown color of the apothecia as observed in our collection is in accord with the interpretation of *O. auricula* sensu Massee. He based his concept of the species in part on the statement made by Schaeffer (1763) who described and illustrated a fungus which Massee interpreted as *O. auricula*. Rehm (1887–1896), Boudier (1905–1910), and Bresadola apply the specific
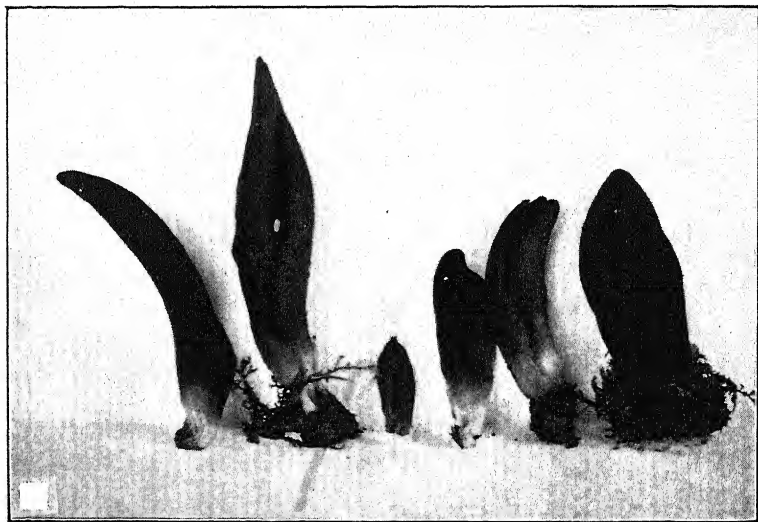
Photo C. H. Kauffman.

FIG. 21. *Otidea auricula.* × ½.

name *O. auricula* to fungi having a whitish exterior. Both Boudier and Bresadola have illustrated such apothecia. Massee stated that the hyphal structures of the two species were different; that the exciple in *O. auricula* was parenchymatic while in *O. neglecta* the outermost cells were arranged parallel. Our North American collections have the dark color accorded to *O. auricula* (Cke.) Massee but have a palisade arrangement of the excipular layer similar to what Massee (l.c.) reported for *O. neglecta.**

* Since this paper went to press the writer has, through the kindness of Sir Edward Salisbury, seen specimens of *O. auricula* and *O. neglecta* from Kew

His illustration shows the palisade layer to be composed of parallel chains, which is unlike the palisade in the American specimens of *O. auricula*. In view of the condition found in the American material, one wonders whether or not Massee's conclusions were an altogether correct interpretation. The spores as reported in the literature are given as large, 22–25 × 12–16 μ. The paraphyses are straight and filiform. In these respects our specimens conform to the European *O. auricula*. Boudier placed the fungus in *Wynnella*.

MATERIAL EXAMINED: G. B. Cummins, Echo Lake, Flathead Nat. Forest, Montana, July 23, 25, and 31, 1928; F. J. Hermann, Big Stone Bay, Emmet Co., Mich., June 13, 1936.

OTIDEA SMITHII Kanouse, Pap. Mich. Acad. Sci. Arts & Letters 24:28. 1939 (FIGS. 8, 9).

Apothecia solitary or cespitose, arising from a large solid foot-like base composed of mycelium intermixed with soil, elongate, ear-shaped, fragile when dry, split on one side, up to 8 cm. in height, outside of apothecium "Van Dyke brown," "Rood's brown" shading to "vinaceous" toward base, inside "wood brown," drying "vinaceous-buff"; hypothecium, composed of hyaline, loosely interwoven hyphae; excipular layer narrow, composed of small thick-walled, brown cells loosely and irregularly arranged, at the surface bunched in shallow piles formed from groupings of 3–5-celled chains, surface cells covered with small granules golden brown in color; asci cylindrical, 100–160 × 12–14 μ, 8-spored, not turning blue in iodine; spores hyaline or faintly colored yellowish, narrowly elliptical, smooth, biguttulate, 10–12 (14) × 6–7 μ; paraphyses with large hooked or bent apices, hooks sometimes ornamented by small, irregular protuberances.

HABITAT: On soil under conifers.

MATERIAL EXAMINED: E. G. Simmons, Lower Tahoma Creek, Wash., Sept. 5, 1948, *2180, 2182;* A. H. Smith, Crescent City, Calif., Nov. 18, 1937, *8843,* Dec. 3, 1937, *9340;* Lower Tahoma Creek, Wash., Sept. 28, 1948, *30994, 30995.*

Herbarium presumably those seen by Massee. The writer could find no appreciable difference in the specimens examined. They appeared to have a pallisade arrangement of the excipular cells. No specimens retained any appearance of white exteriors.

Otidea grandis (Pers.) Rehm. 1887–1896. p. 1023. 1894 (Figs. 11, 12).

Apothecia solitary or cespitose, 1–2 cm. high, 1–4 cm. broad, fleshy-leathery, drying horny, stipitate, truncate, expanded, split to base, edges of split and top of apothecium deeply enrolled when dry; outside "Van Dyke brown," "liver brown" mealy-scurfy, hymenium pale, "vinaceous fawn," "ochraceous tawny" (dry), frequently with patches of red-orange, the orange red color conspicuous when the cups are revived in water, stipe thick, up to 1 cm. long, yellowish in color; hypothecium composed of coarse, hyaline, septate hyphae, loosely interwoven; excipular layer sharply differentiated from the hypothecial layer, composed of thick-walled hyphal segments that resemble small subglobose or irregularly hexagonal cells, walls dark brown, 15–20 μ in diameter, outermost layer of excipular segments covered with minute granules which are subglobose, golden brown in color, becoming easily detached; outside drying mealy, rough; asci cylindrical, 185–200 × 10–12 μ, 8-spored, uniseriate, not turning blue in iodine, collapsing when empty, spores long elliptical to slightly fusoid, 14–17 × 6–7 μ, biguttulate, outer wall smooth, inner wall becoming minutely roughened in age, occasionally biseriate in upper part of asci; paraphyses filiform, frequently forked below, apices strongly hooked, sometimes clavate, 4–5 μ in diameter.

Habitat: On the ground.

Distribution: New York, Nova Scotia, Michigan, Europe.

Discussion: Rehm remarked upon the lack of the green color in *O. grandis*. Boudier (1905–1910) and Bresadola (1932) both illustrate the species as having olive green shades in the exterior of the cups. Our North American collections as well as the European collection represented by Sydow, Mycotheca Germanica No. 2354 lack green color in the dry condition; they are uniformly liver brown. While no color notes are available on fresh material, it is doubtful whether or not any distinct olive green color was ever present in the outside of the apothecia. The hymenium (dry) is lighter in color but when soaked in water developed some shade of orange, frequently a bright orange-red. The apothecia in our material are all more shallow than indicated by Boudier's illustration. In microscopic characters, however, the specimens conform with Rehm's description of the species. The spores are long-elliptical and tend to be narrow at the ends.

The outer wall is smooth but in age the inner wall is minutely roughened. This character is apparent with the aid of an oil immersion lens. The granules on the excipular segments add to the intensity of the brown color of the outside of the apothecia. They are readily washed off in a water mount. Boudier (1905–1910) illustrated them for *O. umbrina,* but no other investigator seems to have observed them for they have not been mentioned elsewhere. They make a useful diagnostic character for these species.

Material examined: Ellis and Everhart, North American Fungi *1778,* second series, as *Peziza onotica* Pers.; Sydow, Mycotheca Germanica *2354;* C. H. Kauffman, Caroline, N. Y., Sept. 6, 1903; Lake Woods, Mich., Aug. 18, 1915; Morten Lange, Burt Lake, Cheboygan Co., Mich., Aug. 4, 1947; E. B. Mains, Deerton, Mich., Sept. 2, 1932, *32–517;* Rock River, Mich., Sept. 6, 1932, *32–609;* Emerson, Mich., Sept. 2, 1933, *33–580;* A. H. Smith, Salmon River, Nova Scotia, Aug. 18, 1931, det. L. E. Wehmeyer (*1344*) as *O. leporina;* Catlin Lake, N. Y., Aug. 19, 1934, *396;* Milford, Mich., July 29, 1937, *6687;* Maple River, Cheboygan Co., Mich., July 22, 1947, *25931.*

Otidea Kauffmanii sp. nov. (Figs. 13, 14).

Apothecia solitaria usque gregaria, stipitata, 2–3 cm. alta, truncata, 2–4 cm. lata, rupta, "chamois" usque ochracea, sicco roseo-lutea; hymenio cremeo-luteo, sicco roseo-luteo; stipite 5–10 mm. alta, 3–5 mm. crassa; hypothecio prosenchymatico, excipulo pseudoparenchymatico, cellulis superficiei irregularibus; ascis 150–200 × 10–12 μ, sporis ellipticis, levibus, biguttulatis, 8–10 (12) × 5–6 (7) μ; paraphysibus hyalinis, saepe subtus ramosis, filiformibus, apicibus subito incrassato, globosis aut clavatis, 6–10 μ diam.

Ad humum, 18 Julii 1915, Lakeland, Michigan, C. H. Kauffman, **Typus.**

Apothecia solitary to gregarious, stipitate, 2–3 cm. in height, truncate, 2–4 cm. in width, split down the short side of the cup, fleshy, hard when dry, "chamois" to "ochraceous" (fresh), dirty pinkish buff (dry); hymenium "cream buff" (fresh), "pinkish buff" (dry), whitish pubescence on lower part of cup extending into the stipe; stipe 5–10 mm. long, 3–5 mm. thick; hypothecium composed of hyaline hyphae, densely interwoven, excipular layer 100–150 μ thick, pseudoparenchymatic, forming irregular piles of cells on the outer surface; asci cylindrical, 150–200 × 10–12 μ, base long, slender, sometimes twisted, 8-spored, not colored blue in iodine; spores elliptical, smooth, biguttulate, faintly colored yellowish, 8–10 (12) × 5–6 (7) μ; paraphyses flexuous, filiform, hyaline, septate, infrequently branched below, apices enlarged into broadly clavate, pyriform or globose heads 6–8 (10) μ diameter, frequently bent (never hooked).

HABITAT: On the ground.

DISTRIBUTION: Michigan.

DISCUSSION: Ample notes were made by Dr. Kauffman on the fresh material of the collection which has been designated as the type. The globose heads of the paraphyses were noted by him in the fresh material. They are also conspicuous in the dry specimens. Paraphyses of this type were also found in *Otidea rainierensis* which is described in this paper. However, the two species are distinct on the basis of size, shape and color of apothecia and also on differences in spore size.

MATERIAL EXAMINED: In low frondose woods, Lakeland, Michigan, July 18, 1915, collected by C. H. Kauffman, **type.** Reported by C. H. Kauffman, Report Michigan Academy of Science 9: 146. 1917, as *Otidea phlebophora* (B. & Br.) Phillips; A. H. Smith and R. J. Porter (Smith *21147*), George Reserve, Pinckney, Mich., Oct. 6, 1945; A. H. Smith, Strawberry Lake, Mich., July 19, 1929.

Otidea rainierensis sp. nov. (FIGS. 15, 16).

Apothecia solitaria aut gregaria, ex basi stipitosa oriunda, 3–7 cm. alta, 3–5 cm. lata, in siccitate fragilia, extus ochraceo-lutea usque pallide brunnea, intus avellanea usque grisea; hypothecio prosenchymatico, excipulo pseudoparenchymatico, catenis brevibus cellularum praedito; sporis ellipticis, levibus, biguttulatis, 10–12 × 6–7 (8) μ; paraphysibus filiformibus, apicibus subito incrassatis, globosis aut subglobosis aut clavatis 6–8 (10) μ diam.

Ad humum sylvaticum. Lower Tahoma Creek, Mt. Rainier National Park, Washington, Aug. 23, 1948. A. H. Smith No. 30553, **Typus.**

Apothecia solitary to gregarious, arising from a short stalk-like base, 3–7 cm. in height, nearly as wide as high, substance thin and fragile when dry, split to the base, edges enrolled, exterior "ochraceous buff," "cinnamon buff" to "wood brown" (dry), inside "avellaneous," "vinaceous buff" to "drab gray" (dry), creamy white toward base, stipe up to 1 cm. in height, tending to be hollow; hypothecium composed of hyaline hyphae loosely interwoven, merging gradually into a shallow excipular layer 50–100 μ in thickness, exciple composed of large subglobose or elongated cells only faintly colored yellow, irregularly arranged, the outermost layer of which produces a few chains; asci cylindrical, 140–160 × 10 μ, frequently with a long slender stalk-like base, not turning blue in iodine; spores elliptical, smooth, faintly yellowish, biguttulate, 10–12 × 6–7 (8) μ, arranged obliquely in the asci; paraphyses hyaline, septate, very slender, filiform, abruptly thickened at the apices into broadly clavate, pyriform, subglobose to globose heads 6–8 (10) μ in diameter.

Habitat: On humus in woods.

Distribution: Washington.

Material examined: Lower Tahoma Creek, Mt. Rainier National Park, Wash., Aug. 23, 1948, A. H. Smith *30553,* type; A. H. Smith, Aug. 22, and Aug. 23, 1948, *30443, 30556;* E. G. Simmons, Sept. 5, 1948, *2179.*

Otidea abietina (Pers. ex Fr.) Fuck.   Symb. Myc. 330.   1869–1870 (Figs. 17, 18).

Apothecia solitary or gregarious, short stipitate, 2–4 cm. broad, 2–3 cm. in height, usually split on one side, truncate, "liver brown" outside, concolorous within, stipe short, arising from a mass of debris bound together with mycelium, whitish tomentose at base; hypothecium composed of coarse hyphae loosely interwoven, excipular layer consisting of large, thick-walled cells hexagonal in shape, the outermost layer of cells smaller in size, and forming a nearly even surface with but few protruding chains of cells; asci cylindrical, 200–250 × 12–15 μ, 8-spored, not colored blue in iodine; spores elliptical, smooth, faintly colored yellowish, biguttulate, 18–20 (22) × 10–12 μ; paraphyses filiform, forked below, septate, apices bent, enlarged and variously ornamented with proliferations and notches or branches usually on the under side of the bent portion, filled with granules faintly colored yellowish, extending beyond the asci forming a loose tangle.

Habitat: On ground.

Discussion: *Peziza abietina* Pers. was transferred to the genus *Otidea* by Fuckel. It has since been placed in *Aleuria* by Gillet (1879), in *Discina* by Rehm (1887–1896). Boudier placed it in his genus *Pseudotis,* Bresadola (1932) in *Otidea,* and Seaver (1928) in *Peziza.* These interpretations are indicative of the uncertainty regarding the generic position of the species. The fact that apothecia are sometimes without a split is probably the reason for its being assigned to genera other than *Otidea.* However, there is sufficient proof that the split condition is the more common, and thus it is logical to seek for the species in the genus *Otidea;* therefore a description is included here. That there is no blue coloration in iodine, that the paraphyses are bent, and that the spores are biguttulate, suggest its affinity with species of *Otidea.* It is certain that one would expect to find it listed in *Otidea* whenever cups with true splits were found and the bent paraphyses

would also suggest this relationship. The under side of the bend in the paraphyses is ornamented with notches and short proliferations which give them a distinctive appearance. It is not uncommon to find similar modification in paraphyses in other *Otidea* species but never has the author seen them so profuse or so conspicuous. The paraphyses project beyond the asci and form loose tangles but are not organized into a definite epithecial layer. Boudier (1905–1910) and Bresadola (1932) illustrate them, but the latter's figures show fewer proliferations. The spore size according to most authors is reported as large (18–20–26 × 10–12 μ). Rehm points out that the specimen cited by Fuckel (No. 1226) which he himself examined is *Peziza badia;* that the spores measured only 14 × 7 μ, and are rough. The notched paraphyses, the lack of blue coloration and split apothecia were constant in the collections cited in this study.

MATERIAL EXAMINED: Sydow: Mycotheca Germanica *2540* (*Discina abietina* (Pers.) Rehm). G. B. Cummins, Echo Lake, Montana, July 6, 1928; C. H. Kauffman, Ithaca, N. Y., Aug. 6, 1904; C. H. Kauffman and D. V. Baxter, Tolland, Colo., Sept. 1920; E. B. Mains, Rock River, Mich., Aug. 20, 1932, *32-179;* A. H. Smith, Lake Quinault, Wash., May 17, 1939, *13566;* Lake Crescent, Wash., Oct. 28, 1935.

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EXPLANATION OF FIGURES

FIG. 1. Paraphyses of *Otidea leporina* var. *typica;* FIG. 2. Spores of *Otidea leporina* var. *typica;* FIG. 3. Paraphyses of *Otidea alutacea* var. *typica;* FIG. 4. Spores of *Otidea alutacea* var. *typica;* FIG. 5. Straight, filiform paraphyses of *Otidea auricula;* FIG. 6. Spore of *Otidea auricula;* FIG. 7. *Otidea auricula;* a fascicle of elongate cells such as form the outermost layer of exciple; FIG. 8. Paraphyses of *Otidea Smithii;* FIG. 9. Spores of *Otidea Smithii;* FIG. 10. Detail showing a branched chain of cells in the exciple of *Otidea onotica;* FIG. 11. Paraphyses of *Otidea grandis;* FIG. 12. Spores of *Otidea grandis;* FIG. 13. Paraphyses of *Otidea Kauffmanii,* showing the abruptly clavate and globose apices; FIG. 14. Spores of *Otidea Kauffmanii;* FIG. 15. Paraphyses of *Otidea rainierensis* showing the clavate to globose apices; FIG. 16. Spores of *Otidea rainierensis;* FIG. 17. Paraphyses of *Otidea abietina* showing the protuberances frequently found; FIG. 18. Spores of *Otidea abietina.*

A NEW SPECIES OF ACHLYA WITH COILED OOGONIAL STALKS [1]

T. W. JOHNSON, JR.[2]

(WITH 2 FIGURES)

In the course of recent studies on the genus *Aplanes,* a considerable number of soil and water samples have been collected from various localities in Michigan. From two of these collections, made in Washtenaw County, a species of *Achlya* has been recovered which differs markedly from any of the hitherto described species of the genus.

Following recovery from the soil, a single-spore isolate was grown on cornmeal agar, then transferred to boiled, split hempseed, placed in 30 cubic centimeters of sterile, charcoal-filtered, distilled water, and kept at a temperature of 22° C. The description of the species was compiled from such cultures. An examination of the isolate revealed that whereas it resembled, in certain general features, several of the papillate species of *Achlya,* it was actually quite distinct from them. These distinctions are found in the characteristic coiled, spring-like oogonial stalks, the large oospores, the variously-shaped oogonia, and in the sparseness of antheridia. For these reasons, it is considered a new species.

Achlya spiracaulis sp. nov. Myceliis in semine *Cannabis sativae* tenuibus, hyphis ramosis porrectis usque ad 3.0–4.2 cm. in diametrum. Gemmis paucis longis quandoque inaequalibus; sporangiis copiosis, attenuatis sine cylindraciis ad basim saepius latioribus, 280–637 μ longis, 28–42 μ in diametrum, plerumque 497–595 × 35–42 μ, e basi proliferantibus et tunc cymosis; zoosporiis 11.0–14.1 μ in diametrum, apice dehiscentibus et in sphaerula dispositis, perraro *in situ* germinantibus. Oogoniis copiosis in ramulis lateralibus longis et spiriformibus aut raro brevibus et rectis, nonnumquam terminalibus in summo hyphae spiriformis, saepius autem. Oogoniis ipsis

44.0–83.6 μ in diametrum sine spinis, plerumque 60.6–72.5 μ, aut globosis aut variis, tunica crassa non-punctulata, consista spinis sublongis obtusis. Oosporiis numero 1–12, plerumque 4–8, globosis 13.2–49.5 μ in diametrum, plerumque 25.0–30.8 μ, guttulis oleosis centrice dispositis; tunica crassa, hyalini. Antheridiis paucis diclinibus aut androgenibus.

Hab: ad terram humosam in ripa rivi intermittentis, Nichols' Arboretum, University of Michigan, Aprilis 4, 1949.

Mycelial growth tenuous, extensive, the colony reaching a diameter of 3.0–4.2 cm. on hempseed; principal hyphae up to 119 μ in diameter at the base; usually sinuous, moderately branched. Gemmae few, single, terminal, long-tapering and sporangium-like; occasionally quite irregular; upon germination forming thin hyphal branches usually bearing small apical sporangia; rarely germinating to form a terminal oogonium. Sporangia abundant, terminal, long-tapering or cylindrical, usually broadest at the middle or near the base, 280–637 μ long by 28–42 μ in diameter, predominantly 497–595 × 35–42 μ; secondary sporangia arising by cymose branching or in basipetal succession from below the primary sporangia, in which case including basally a portion of the hypha. Spores at discharge collecting in a hollow sphere at the apical pore, or germinating *in situ* in some secondary sporangia; encysted spores 11.0–14.1 μ in diameter. Oogonia abundant, borne laterally on very long, tightly or loosely coiled and bent stalks, rarely on short, straight or bent stalks; stalks sometimes branched, and bearing 2–3 oogonia; infrequently terminal, with the hyphal branch also coiled; never observed intercalary; varying greatly in shape, mostly spherical or ovoid, occasionally barrel-shaped, oblong or irregular; wall averaging 1.2 μ thick, unpitted, densely studded with short or long, round-pointed spines 4.4–30.8 μ in length, averaging 9.9–14.3 μ; oogonia, not including spines, 44.0–83.6 μ, averaging 60.6–72.5 μ in diameter; irregular oogonia averaging 104 × 51 μ. Oospores 1–12, mostly 4–8; 13.2–49.5 μ, averaging 25.0–30.8 μ in diameter; centric, with a single layer of oil droplets completely surrounding the protoplasm; typically spherical, but occasionally block-shaped or ellipsoidal from pressure; wall thick, hyaline. Antheridial branches present on 8–12 per cent of the oogonia, about equally androgynous and diclinous; antheridia applied by their apices to the oogonia; antheridial cells rarely formed; fertilization tubes not observed.

From soil, along bank of an intermittent stream, Nichols' Arboretum, University of Michigan, April 4, 1949 (**type**), and from soil, along bank of Fleming Creek, near Geddes Road, Washtenaw County, Michigan, May 8, 1949.
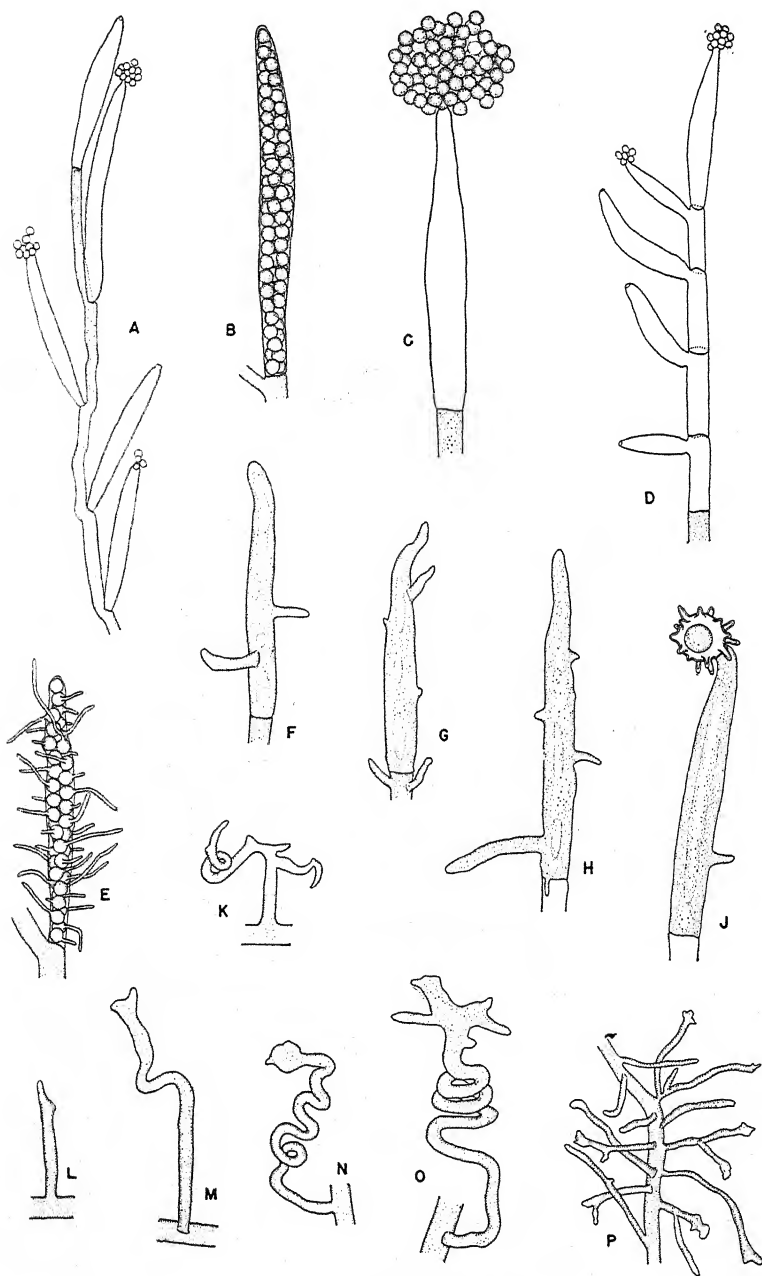
FIG. 1. *Achlya spiracaulis.*

Slides of preserved material from the type culture are being deposited in the herbaria of the University of Michigan and the University of Illinois.

Achlya spiracaulis has been compared with the original descriptions of all known species of *Achlya,* and although it embodies characteristics of several of these, it differs from them in one outstanding respect: *i.e.,* the production, even under varying environmental conditions, of coiled oogonial stalks. In this feature, the fungus resembles *Achlya contorta* Cornu, but from Cornu's incomplete description and meager illustrations (8), his species possesses smooth-walled oogonia. On the basis of oospore number, and unpitted, spiny oogonial wall, there is a resemblance to *Achlya papillosa* Humphrey (10). However, the sparingly developed sporangia, short oogonial stalks, and the abundant though imperfectly formed antheridia immediately separate *A. papillosa* from the present species.

Coker (3) reports coiling oogonial stalks for *Achlya proliferoides* (3, pl. 36, *fig. 6*), but because of its smooth oogonial walls, short oogonial stalks, and abundant antheridia, his species is obviously not the Michigan isolate. Coiled oogonial stalks were also reported by Coker (3) for *Achlya Orion,* but these were formed only at a temperature of 36° C. Further, the smooth-walled oogonia and the smaller number of larger oospores also distinguish *A. Orion* from *A. spiracaulis.*

The original description of *Achlya recurva* by Cornu (8) is rather vague. A more complete study was made by Latham (11) of an isolate of *A. recurva* found in North Carolina. *Achlya spiracaulis* evidently resembles Latham's fungus in certain respects, particularly with reference to the atypical, contorted oogonia, the size of encysted zoospores, oospore number, and the scarcity of gemmae. However, because of the coiled oogonial stalks, larger oogonia, larger oospores, and smaller percentage of antheridia, *A. spiracaulis* is distinct from *A. recurva.*

With the exception of *Achlya contorta,* coiled oogonial stalks are not reported for any of the European species of *Achlya.* In addition, other characteristics of these species, such as oospore size and number, size of oogonia, and antheridial characteristics, differentiate them from *A. spiracaulis.*

OBSERVATIONS

It would be repetitious to record details of sporangial formation and subsequent development and discharge of the zoospores, since, with the possible exception of basipetalous development of secondary sporangia (FIG. 1, D) in older cultures, and germination *in situ* in many of these secondary sporangia (FIG. 1, E), the developmental morphology of the sporangia is that characteristic of the genus *Achlya* (12). Certain points concerning oogonial development in *A. spiracaulis* should, however, be mentioned. As observed in young cultures in sterile, charcoal-filtered, distilled water, the oogonium first appears as a short, lateral protrusion from the main hypha (FIG. 1, L). Over a three or four day period following the appearance of the protrusion, there is a marked elongation into a definite lateral stalk, which, after about three days, begins the characteristic coiling (FIG. 1, M, N). Branching of the oogonial stalk may occur prior to or immediately after coiling (FIG. 1, K). Upon completion of coiling, the tip of each stalk enlarges, and one to several spine-like protrusions make their appearance, which give to the oogonial initial a distinct irregular shape (FIG. 1, 0). The tip continues to enlarge, the spines become more numerous, and ultimately the oogonium reaches mature size. It is at about this time that antheridia, if any, are formed. The development of oospores is slow, even in oogonia which are apparently fertilized, since in most cases no oospores are visible for six to eight days after the oogonia reach mature size. Where oogonia are formed terminally, the first indication of their development is again coiling of the stalk, in this instance, the hyphal tip. The subsequent development of terminal oogonia is similar to those formed laterally.

In fifteen- to twenty-day-old cultures, there are occasional instances of oogonia borne on bent or once-coiled stalks (FIG. 2, G, J). Many of the later-formed stalks do not bear oogonia, and in still older cultures may give the hyphae a distinctive, short-branched, bushy or irregular appearance (FIG. 1, P).

Preliminary qualitative studies on the effect of variations in environments other than pure water on *Achlya spiracaulis* have been undertaken. Results would seem to indicate that the distinguishing
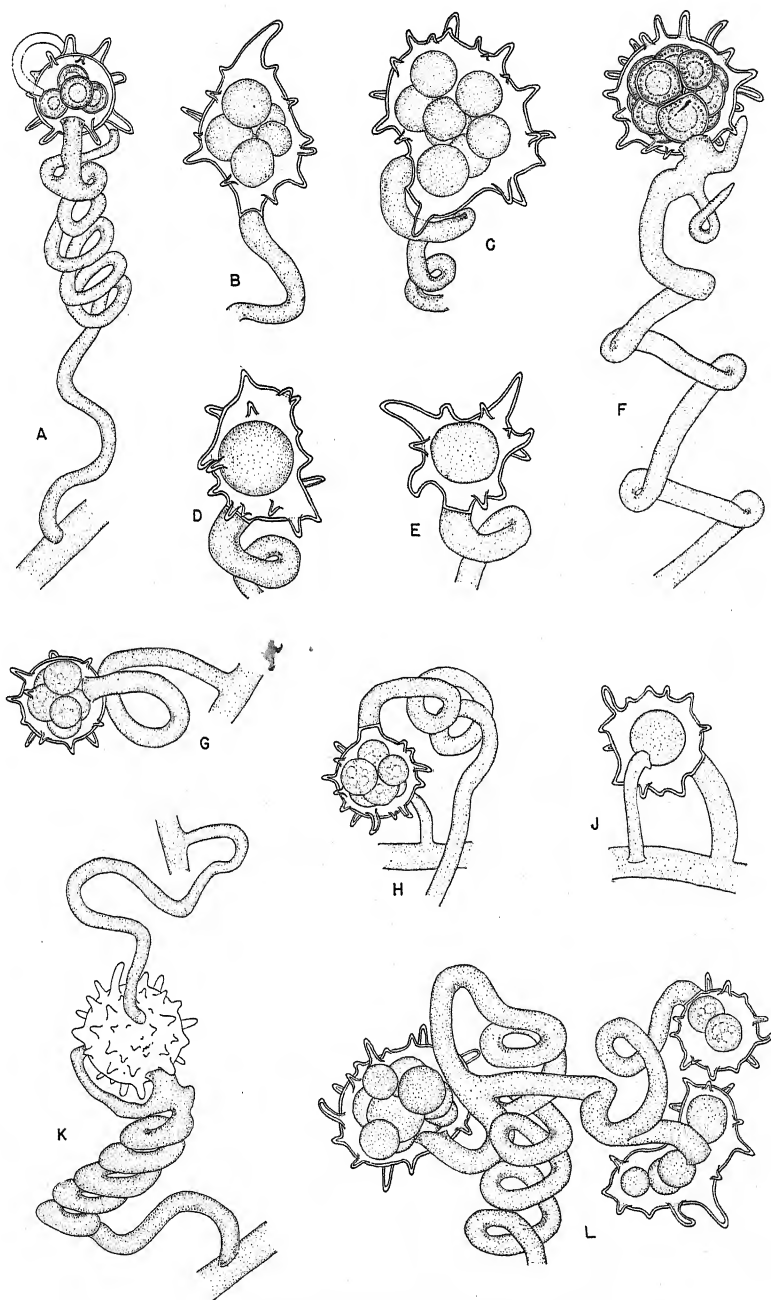
FIG. 2.   *Achlya spiracaulis.*

coiled-stalk characteristic of the fungus, as well as such features as origin and size of the oogonia, papillations of the oogonial wall, and percentage of antheridia, are consistently stable characteristics.

To determine the characteristics of the fungus when grown in a more natural habitat, a series of 20 single spore isolates were grown in 20 separate samples of water containing leaves and other debris. The water and debris samples were selected from several different sources such as fresh cold water, stagnant cold water, and warm, clear, running water. After the colonies had developed for two weeks in these "natural" habitats, they were observed for possible changes in morphology. In all of these cultures, the fungus retained the characteristics as described. Of interest, nevertheless, is the fact that the appearance of oospores in these cultures was remarkably slow, in most cases not occurring for two to three weeks after the formation of the oogonia.

A more complete study of the effect of varied environments on the morphology of the fungus is to be undertaken at a future date.

SUMMARY

A new species of *Achlya,* from Michigan, is described as *Achlya spiracaulis.* The fungus is characterized by long, tightly or loosely coiled oogonial stalks, by which it is easily distinguished from all other species of *Achlya;* large, papillate, spherical to irregular oogonia; large, centric oospores; unpitted oogonial walls, and a low percentage of about equally diclinous and androgynous antheridia.

The fungus was grown under several varied environmental conditions (including those simulating natural ones), to induce changes in the distinguishing morphological characteristics, but the results of these preliminary studies show that the coiled oogonial stalks, origin of oogonia, and frequency and type of papillations are consistently stable.

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DESCRIPTION OF FIGURES

Achlya spiracaulis. Fig. 1, *A.* Habit of sporangia, showing cymose branching. *B.* Undischarged sporangium. *C.* Discharged sporangium. *D.* Habit, showing basipetalous development of secondary sporangia. *E.* Secondary sporangium with spores germinating *in situ. F, G, H.* Typical gemmae. *J.* Gemma which has germinated to form a small oogonium at its apex. *K.* Young oogonial stalk which shows branching prior to coiling. *L, M, N, O.* Stages in the development of the oogonial stalk, and rudiment of the oogonium. *P.* Cluster of undeveloped oogonial stalks in a thirty-day-old culture. All drawings made with aid of Spencer camera lucida. Figs. *A, D, F, G, H, J,* and *P,* × 170; all others, × 410.

Fig. 2, *A.* Typical spherical oogonium with long, coiled, lateral stalk, androgynous antheridium, and mature oospores. *B, C, D, E.* Irregular, contorted oogonia with immature oospores. *F.* Terminal, spherical oogonium showing loose-coiling of hypha, mature oospores, and an undeveloped antheridium. *G.* Spherical oogonium with once-coiled, lateral stalk. *H.* Spherical oogonium with diclinous antheridium and short-coiled stalk. *J.* Spherical oogonium with androgynous antheridium and short, bent, lateral stalk. *K.* Spherical oogonium showing tightly-coiled lateral stalk, papillations of oogonial wall, and both a diclinous and an androgynous antheridium. *L.* Coiled, branched, lateral oogonial stalk with one spherical, one oval, and one constricted oogonium, all with immature oospores. All figures × 840.

STUDIES ON SOME UNUSUAL HETERO-BASIDIOMYCETES FROM WASHINGTON STATE [1]

GEORGE NYLAND [2]

(WITH 6 FIGURES)

In a previous paper (6) the morphology and cytology of an undescribed Heterobasidiomycete was discussed. It was noted that, although the fungus had a spore stage indistinguishable from *Sporobolomyces,* it could not be placed in that genus because of the presence of mycelium with numerous clamp connections and with abundant, thick-walled, brown chlamydospores.

This paper describes in more detail the characteristics of the fungus and, to accommodate it, a new genus and species, *Sporidiobolus Johnsonii,* is proposed. A previously undescribed species of a closely related genus, *Itersonilia* Derx, is also described.

Sporidiobolus gen. nov.

Ballistosporae in sterigmatibus formatae et per vim effusae. Sterigmata aetheria e mycelio aut statim e ballistosporis ascendentia. Ballistosporae ad propagationem multiplicatione sporarum aut ad gemmandum eodem modo fermenti aptae. Mycelium hyalinum septatum cum conjunctionibus. Chlamydosporae spisse circumdatae et fuscae, terminales aut intercalares.

Species typica: *Sporidiobolus Johnsonii.*

Ballistospores produced on tips of sterigmata of variable length and forcibly abjected by drop-excretion mechanism. Sterigmata arising as branches from mycelium or directly from ballistospores. Ballistospores capable of reproduction by repeating spores or by budding in yeast-like manner. Mycelium septate with clamp connections. Chlamydospores terminal or intercalary, brown, thickwalled, produced on mycelium.

Sporidiobolus Johnsonii spec. nov.[3]

Ballistosporae 6–13 × 3.5–6.5 μ (sporae circa 9 × 5 μ sunt), hyalinae, quae per saturam puniceae, leve flexae, incongruentes, reniformiatae-lunatae sunt, quae propagatione crescendarum sporarum aut cellis quae eodem modo fermenti gemmant, quae aut in re gemmandis perseverare aut ballistosporas parere possunt, germinant. Ballistosporae e mycelio parere etiam possunt. Mycelium linea media 1.5–3 μ septatum, copiosis cum conjunctionibus. Chlamydosporae maturitate fulvae, linea media 10–16 μ, in brevibus hyphis formatae, terminales aut intercalares, cum conjunctione in caule in cuiusque sporae radice.

Hab. In soro *Phragmidii rubi-idaei* (DC.) Karst. in foliis *Rubi idaei* L. Puyallup, Washington, U. S. A.

Ballistospores 6–13 × 3.5–6.5 μ (ave. 9 × 5 μ), hyaline by transmitted light, salmon pink in mass, slightly curved, asymmetrical, reniform-lunate; germinating by forming sterigmata and repeating ballistospores, or by budding vegetative, yeast-like cells which in turn may continue to bud or may produce ballistospores. Ballistospores may also be produced from the mycelium on short sterigmata. Colony on malt agar appressed, hyphae 1.5–3 μ diameter, septate, with clamp connection at almost every septum. Chlamydospores hyaline when young, golden brown when mature, 10–16 μ diameter (ave. 12 μ), wall 1–1.5 μ thick, contents oily, formed on hyphae in or on surface of matrix, usually on short lateral stalks, terminal or intercalary, with a clamp connection on each stalk at base of spore, often with a hyaline hyphal projection from distal end of chlamydospore.

Habitat: In pustule of *Phragmidium rubi-idaei* (DC.) Karst. on living leaf of *Rubus idaeus* L. vicinity of Puyallup, Washington, summer 1946.[4]

This species differs from species of *Sporobolomyces* only by its production of a well developed mycelium and chlamydospores. The budding, vegetative cells and the ballistospores are indistinguishable from those occurring in species of *Sporobolomyces*.

During the course of this work several other fungi resembling *Sporidiobolus* were isolated and one of these is described below as a new species in the genus *Itersonilia* Derx.

The genus *Itersonilia* was proposed by Derx (3) in May, 1948, to accommodate a fungus isolated by him for the first time in 1925. He obtained the fungus by attaching a leaf of *Althaea rosea* bearing

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[3] Type specimen deposited in the Mycological Herbarium, State College of Washington, Pullman, Washington.

[4] Isolated by Dr. Folke Johnson and named in his honor.

numerous sori of *Puccinia malvacearum* Mont. to the lid of a petri dish. Of the many basidiospores of the rust that were projected downward onto the agar, he observed that the majority showed only the beginnings of a germination which ended with a sketchy secondary spore. But among the number some produced a true mycelium equipped with numerous clamp connections. From the mycelium there developed sparse aerial hyphae, and from these were produced long, tapering sterigmata on which were produced spores having the characteristic form of basidiospores. These were projected at maturity and continued the same cycle of development. The spores of this fungus were nearly the same size as those of *Puccinia malvacearum* so unless permitted to grow could not easily be distinguished from them. Derx (3) figures enlarged swollen cells with thin cell walls, each provided with a clamp at its base, on the mycelium of *Itersonilia*. He aptly calls his new species *I. perplexans*. Because his descriptions occur in a somewhat obscure journal they are repeated here for convenience (3).

"*Itersonilia* Derx *gen. nov.*

"Mycelium hyalinum, septatum, repens, fibuligerum ut typice in Basidiomycetibus; inflationibus terminalibus denique post excrescentionem terminalem saepe intercalaribus; sporophoris in aera ascendentibus, non-ramosis, apicem versus sensim attenuatis, in sterigma exeuntibus. Sporae solitariae, terminales, asymmetricae, uno latere plus minusve depressae, crassiusculae, non-falcatae, leves, hyalinae.

"Species typica: *Itersonilia perplexans* Derx *spec. nov.*

"*Itersonilia perplexans* Derx *spec. nov.*

"Mycelium 3–5 μ diam., in septis omnibus fibuligerum; inflationibus lato-ellipsoides vel obovoideis, 12–16 × 9–10 μ, hyalinis. Sporae subreniformes vel ovoideae, leves, tenuiter tunicatae, 14–15 μ × 8–10 μ—In foliis *Althaeae roseae* intra soros *Pucciniae malvacearum.*"

The term "ballistospore" has been used by Derx (3) at the suggestion of Dr. M. A. Donk to include those spores of the Basidiomycetes that are forcibly abjected at maturity by the drop-excretion mechanism studied by Buller (1). The general term seems appropriate, especially for the Sporobolomycetaceae and related forms, since it has not been established that the ballistospores in these fungi are actually basidiospores.

A species of *Itersonilia* was obtained by the writer, using essentially the same technique as Derx, from a dead leaf of *Acer macrophyllum* Pursh, in February, 1948. It is considered to dif-

fer sufficiently from *I. perplexans* to warrant the erection of a new species.

Itersonilia pyriformis spec. nov.[5]

Ballistosporae incongruentes, reniformatae-lunatae, 11.7–19.5 × 6.5–9.1 μ (sporae circa 15.6 × 7.3 μ sunt) in sterigmatibus e mycelio aut multiplicatione formatae. Hyphae aetheriae, copiosae, candidae linea media 2.4–2.6 μ, hyphae hyalinae submergitae linea media 1.2–1.5 μ ambae septatae cum in prope quoque septo conjunctionibus. Chlamydosporae in matrice hypharum, hyalinae, subtile circumdatae, aut pyriformatae aut globosae aut ovatae, sporae circa 16 × 7.8 μ sunt.

Hab. In foliis morto *Aceris macrophylli* Pursh.

Ballistospores asymetrical, reniform-lunate, 11.7–19.5 × 6.5–9.1 μ (average 15.6 × 7.3 μ), formed on sterigmata from the mycelium, or by repetition, but not budding in yeast-like manner. Mycelium on malt agar white, aerial, surface hyphae, 2.4–2.6 μ diameter, submerged hyphae hyaline, 1.2–1.5 μ diameter, septate, with clamp connections on almost every septum. Chlamydospores formed on hyphae in matrix, hyaline, thin-walled, pyriform to globose or oval, usually intercalary, average 16 × 7.8 μ.

Habitat: Dead leaf of *Acer macrophyllum* Pursh, collected near Kent, Washington, U. S. A., February 26, 1948, by G. W. Fischer.

This species differs from *Itersonilia perplexans* in that the chlamydospores have no clamp connections associated with them and by the presence of abundant aerial mycelium on malt agar. Also the ballistospores of *I. pyriformis* are somewhat shorter than those of *I. perplexans*. The genus *Itersonilia* differs from *Sporidiobolus* in several ways. In *Sporidiobolus* the ballistospores may bud in a yeast-like manner but in *Itersonilia* they germinate directly to form a mycelium or occasionally form spores by repetition. The chlamydospores in *Sporidiobolus* are brown with thick walls whereas in *Itersonilia* they are hyaline with thin walls.

LIFE HISTORIES, PHYSIOLOGY, AND CYTOLOGY

I. Sporidiobolus Johnsonii

Life History. The life history of *Sporidiobolus Johnsonii* has been described in a previous paper (6). A brief statement was

[5] Type specimen deposited in the Mycological herbarium, State College of Washington, Pullman, Washington.

made regarding germination of the chlamydospores after incubation at 35° C. for 6 days. Actually 12 to 14 days were required because the cultures were removed for 24 hours on alternate days. During the course of the sampling, numerous hyaline chlamydospores were observed to germinate directly by producing germ tubes that developed into a mycelium. These were judged to be young, hyaline chlamydospores and not rounded up ballistospores or vegetative budded cells because in most cases fragments of the mycelium could be observed attached to the spores.

When dilution transfers were made from old cultures, two types of colonies regularly resulted. The original mucous type formed
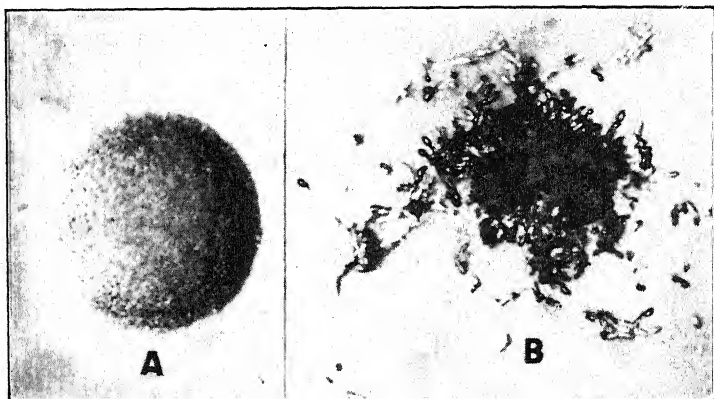
Fig. 1. *Sporidiobolus Johnsonii*. A, mucous and B, non-mucous type colonies. Note ballistospores on sterigmata in B. Approx. × 200.

as a result of budding and a non-mucous type formed as the result of the production of long sterigmata and pseudomycelium consisting of elongated, vegetative, budded cells. Both types of colonies were formed from either vegetative, budded cells or from ballistospores. This phenomenon was also reported as occurring in *Sporobolomyces* by Derx (2). The appearance of the two types of colonies is shown (Fig. 1). The non-mucous type of colony was more abundant if transfers were made from the oldest portion of the culture.

Longevity of this species in culture exceeded 18 months after which sampling was discontinued. Viable ballistospores were pres-

ent as long as the cultures were not completely desiccated, after which time the new growth seemed to arise only from the mycelium. Both ballistospores and new mycelium were produced in every case.

Temperature Requirements. To determine cardinal temperatures, 250 cc. Erlenmeyer flasks containing 35 cc. of 2 per cent Difco potato dextrose agar were inoculated in quadruplicate with 6 mm. disks of agar inoculum containing mycelium and resting spores. Inoculum was prepared on potato dextrose agar in Petri dishes and after thirteen days disks were cut out from the margins
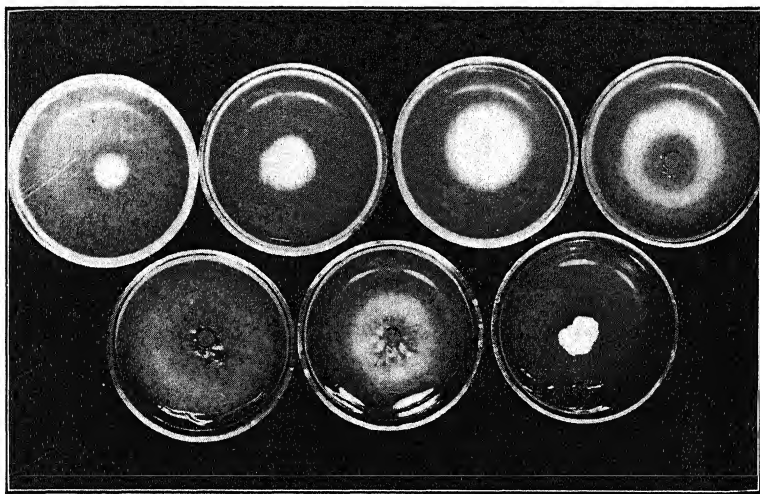
FIG. 2. *Sporidiobolus Johnsonii.* Final measurements after 18 days' growth at indicated temperatures.

of the colonies with a 6 mm. cork borer. Inoculum disks were transferred to the surface of the agar in the flasks with a flat-pointed needle. The flasks were placed in a constant temperature incubator at 26° C. for 17 hours prior to being incubated at the various temperatures, 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. Measurements were made on the 5th, 9th, 13th and 18th days after incubation was begun. Cultures incubated at the optimum temperature (25° C.) completely covered the surface by the 18th day. The results are shown graphically (FIG. 2). Maximum growth of 80 mm. resulted at the optimum temperature of 25° C. Minimum

temperature for growth was approximately 5° C. and maximum temperature at which normal growth could take place was approximately 30° C. Some abnormal growth occurred at 35° C.

At 5° C. there was very little growth, the mycelium was profusely branched, very much appressed, and appeared somewhat mucose. Neither chlamydospores nor ballistospores were observed. At
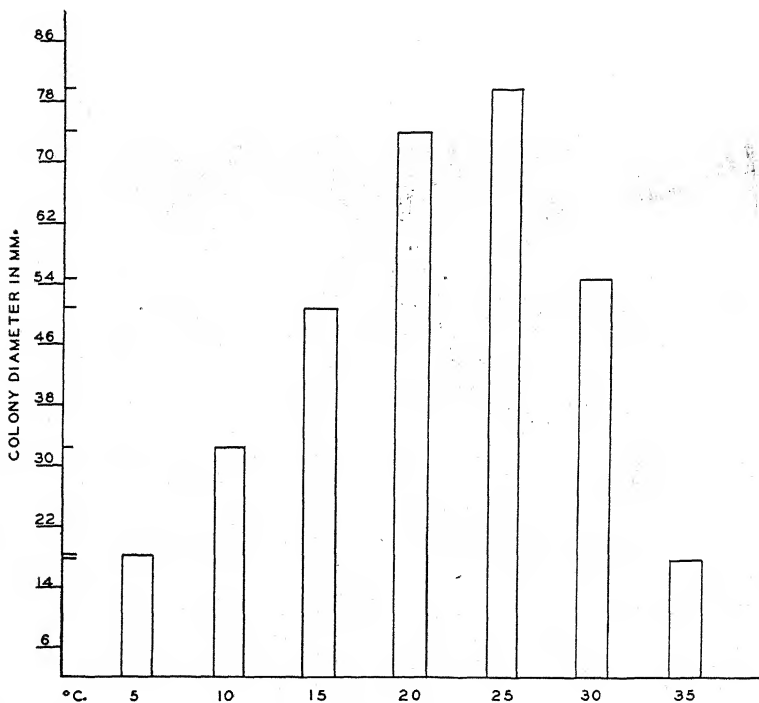
Fig. 3. *Sporidiobolus Johnsonii.* Reaction to various temperatures. Left to right, 5°, 10°, 15°, 20°, 25° (optimum), 30°, 35° C.

10° C. the colony appearance was much the same as at 5° C. but of somewhat greater diameter. Masses of chlamydospores were formed in the normal way but all were hyaline. At 15° C. there was a slight darkening or browning around the inoculum disk due to the brown walls of numerous chlamydospores. At 20° C. brown-walled chlamydospores were formed out from the center of the colony over about half the radius. A few ballistospores were

observed on sterigmata formed on the mycelium on the surface of the colony. At 25° C., the optimum temperature, chlamydospores were observed out to the very margin of the colony and even the margin had a brownish cast indicating rapid pigmentation of the spores after formation. Numerous ballistospores were observed. At 30° C. the margin of the brown, central area was irregular with dark streaks (chlamydospores) extending out almost to the margin of the colony. Some ballistospores were observed. At 35° C. the colony consisted entirely of ballistospores and budded vegetative cells formed in a pinkish, crusty mass. Examination under the microscope revealed that the ballistospores and vegetative cells were germinating to form secondary spores on long sterigmata in addition to budding. No true mycelium or chlamydospores were observed beyond the margin of the inoculum disk. These temperature reactions are illustrated graphically (FIG. 3).

Nutrient Requirements. The comparative rate and type of growth of *Sporidiobolus Johnsonii* was studied on eight agar media, as follows:

1. CD, Czapek's [6] with dextrose, 30 gms./liter
2. CS, Czapek's with sucrose, 30 gms./liter
3. CDP, Czapek's with dextrose plus peptone, 5 gms./liter
4. CSP, Czapek's with sucrose plus peptone, 5 gms./liter
5. C, Cornmeal agar (Difco)
6. P, Prune agar (Difco)
7. PD, Potato dextrose agar (Difco)
8. M, Malt agar (Difco)

Each agar medium was used in quadruplicate in 250 cc. Erlenmeyer flasks containing 25 cc. of medium. All were allowed to age two weeks before inoculation with 6 mm. agar blocks containing mycelium and chlamydospores. It was found that if the agar was allowed to age and dry out, somewhat more uniform mycelial growth resulted. Also aged agar inhibited the excessive formation of masses of vegetative budded cells and ballistospores. The 32 flasks were incubated at 25–26° C. for 15 days and colony measurements taken every other day. The results are presented graphically (FIG. 4). The organism grew most rapidly on malt agar

[6] Basic formula for Czapek's agar: $MgSO_4$ .5 gm., KH_2PO_4 1.0 gm., KCl .5 gm., $FeSO_4$ tr., $NaNO_3$ 2.0 gm., agar 25.0 gm., distilled H_2O 1000 ml., and dextrose, sucrose, and peptone as indicated.

and most slowly on Czapek's with sucrose plus peptone. It apparently utilizes dextrose more efficiently than sucrose for mycelial growth as shown by the difference of 8 mm. in average diameter of the colonies. Dextrose seems to be responsible for the lavish production of budded cells and ballistospores which occurred on Czapek's with dextrose, since this does not occur on Czapek's with sucrose. Peptone also seems to stimulate vegetative budding
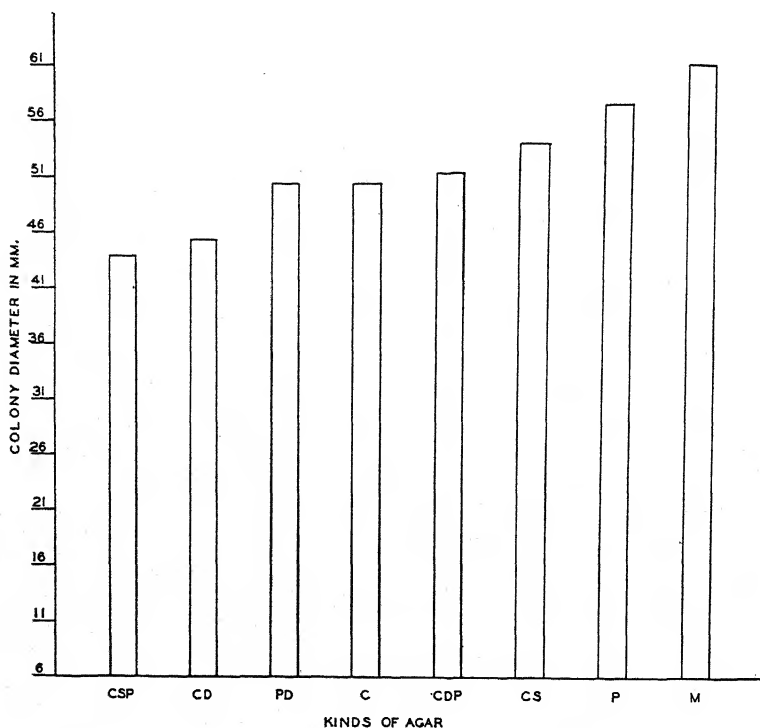
FIG. 4. *Sporidiobolus Johnsonii*. Colony diameter after 15 days' growth on 8 different media.

but to a lesser degree than dextrose. Czapek's with dextrose plus peptone resulted in larger colonies than Czapek's with dextrose alone. Czapek's with sucrose plus peptone, however, resulted in smaller colonies than Czapek's with sucrose alone. In Czapek's agar peptone, apparently, has a depressing effect on growth when associated with sucrose but a stimulatory effect when associated with dextrose, as contrasted to Czapek's with dextrose or sucrose

alone. Figure 5 shows the appearance of the colonies on the various media.

CYTOLOGY. It was shown that the ballistospores and the vegetative yeast-like cells of *Sporidiobolus Johnsonii* are uninucleate. Occasionally binucleate ballistospores were observed (6).

Because of the resemblance of this species to species of *Sporobolomyces*, it was considered advisable to examine as many species of *Sporobolomyces* as possible, cytologically and morphologically. The following available species [7] were studied:

Sporobolomyces alborubescens Derx, *S. gracilis* Derx, *S. odorus* Derx, *S. pararoseus* Olsen and Hammar, *S. roseus* Kluyver and van Niel, *S. pollaccii* Vernona and Ciferri, *S. rubicundulus* (Okunuki) Verona and Ciferri, *S. salmoneus* Derx, *S. salmonicolor* Kluyver and van Niel, *S. salmonicolor* var. *polymyxa* Kluyver and van Niel, *S. shibatanus* (Okunuki) Verona and Ciferri, *S. tenuis* Kluyver and van Niel (species strain Lederer), *Bullera alba* (Hanna) Derx (= *Sporobolomyces albus* Hanna).

All of the above species were observed to have uninucleate vegetative cells and ballistospores, thus confirming the observations of Guilliermond (4) and Buller (1) who studied several of the species.

To obtain mycelium and chlamydospores of *Sporidiobolus Johnsonii* satisfactory for cytological study small blocks of agar were taken from the margins of actively growing colonies on malt agar and transferred to slides containing Mayer's adhesive. The slides were then placed in Petri plates on moist filter paper. Hyphae grew out from the blocks of agar onto the slides and formed resting spores there. After five to ten days the material was killed and fixed by exposing it to fumes of osmic acid in Fleming's weaker killing fluid. The preparations were then permitted to air-dry thoroughly. After drying, a sharp scalpel was used to sever the hyphae from the blocks of agar. When the slides were placed in the bleaching solution the agar blocks swelled and loosened, leaving only the radiating hyphal strands and chlamydospores on the slide, all in one plane. The standard iron-alum-haematoxylin method was used for staining the nuclei.

[7] Cultures of species of *Sporobolomyces* and *Bullera* were obtained from the Centraalbureau, Yeast Division, Delft, Holland.

It was found that mycelial cells were predominately binucleate, especially during earlier stages of growth. The chlamydospores at first are uninucleate but become binucleate upon formation of the clamp connection on the stalk at the base of the chlamydospore. The two nuclei fuse immediately and the spore assumes the characteristic golden-brown color. Attempts to stain the fusion nucleus in mature spores after complete pigmentation had occurred were not successful. Evidently, special treatment is required to remove
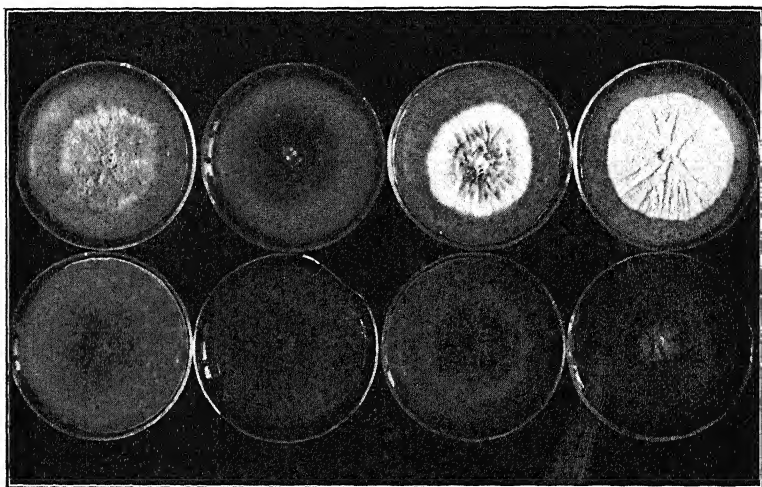
FIG. 5. *Sporidiobolus Johnsonii.* Reaction to various agar media. Upper left to right: Czapek's plus dextrose, Czapek's plus sucrose, Czapek's plus sucrose plus peptone, and Czapek's plus dextrose plus peptone. Lower left to right: Cornmeal, prune, potato dextrose, and malt agars.

oily materials within the spore before the nucleus can be stained in fully pigmented spores.

II. ITERSONILIA PYRIFORMIS

Life History. On nutrient media the ballistospores of *Itersonilia pyriformis* germinate directly to form a mycelium provided with clamp connections at almost every septum (FIG. 6, B). Starting from a single ballistospore, visible colonies are formed within two days at room temperature. Within three or four days ballistospore production begins. The spores can easily be recognized

lying on the agar surrounding the margin of the advancing mycelium. By observing plate cultures under the microscope at low magnification they can also be observed attached to sterigmata which are produced as branches from the mycelium.

The young colonies are raised, rounded, and pure white due to the production of abundant, hyaline, aerial hyphae. As the colony becomes older, the lower surface becomes buff-colored to light tan on malt agar and surface growth becomes appressed.
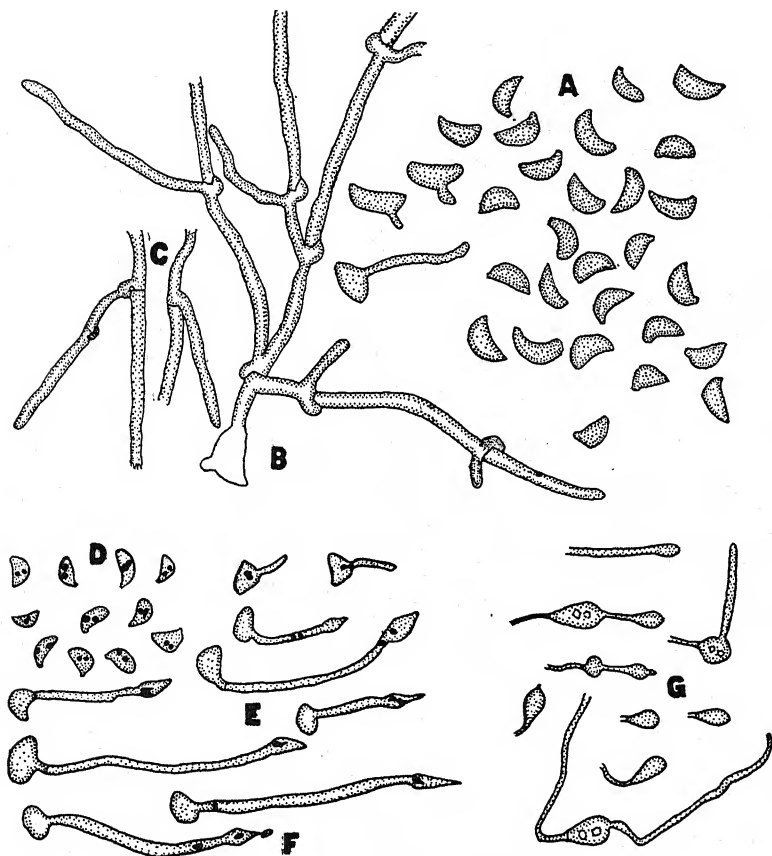
FIG. 6. *Itersonilia pyriformis*. A, Ballistospores; B, Germinating ballistospore showing mycelium and clamps; C, A typical branching arising from clamps; D, Binucleate ballistospores; E, Germinating ballistospores on slide coated with Mayer's adhesive; F, Formation of repeating spore; G, Chlamydospores. Drawn with the aid of a camera lucida. A, B, C, approx. × 500. D, E, F, G, approx. × 350.

When ballistospores germinate on slides that have been coated with Mayer's adhesive, they produce long germ tubes, usually unbranched, each with a swollen spear-point vesicle at the tip. The two nuclei in each ballistospore migrate through the germ tube into the vesicle (FIG. 6, E). Sometimes the germ tube continues vegetative growth but in other cases a secondary ballistospore is formed at the pointed tip of the vesicle (FIG. 6, F) and this spore receives the entire contents of the germ tube, including the two nuclei. Direct production of ballistospores is relatively uncommon on slides containing Mayer's adhesive.

When ballistospores germinate on water agar, either secondary ballistospores are formed directly from the primary ballistospores on short sterigmata, as in *Sporobolomyces* and *Sporidiobolus Johnsonii,* or germ tubes are produced that penetrate downward into the agar. The protoplasm of the germ tube migrates and remains at the growing tip, resulting in complete evacuation of the spore and most of the germ tube. Crosswalls are laid down immediately behind the migrating protoplasm. No clamp connections have been observed under these conditions. On nutrient agar there may be some protoplasmic migration in the germ tube but the tube soon branches and produces a normal mycelium with clamp connections. Germination of the chlamydospores of this species was not observed.

CYTOLOGY. The ballistospores of this species are binucleate in contrast to those of *Sporidiobolus Johnsonii* and those of species of *Sporobolomyces* (FIG. 6, D). Upon germination on nutrient agar they give rise to binucleate mycelia. The nuclei are usually closely associated in the mycelial cells. As far as could be determined the chlamydospores (FIG. 6, G) at maturity are uninucleate, probably containing a diploid fusion nucleus. Considerable difficulty was experienced in getting good preparations of stained chlamydospores.

<center>DISCUSSION</center>

When young cultures of *Sporidiobolus Johnsonii* are observed before mycelial production begins, they cannot be distinguished from some species of *Sporobolomyces*. The masses of ballistospores are pink in color; yeast-like cells are formed and bud exactly as they do in *Sporobolomyces*. Also, the production of bal-

listospores by repetition occurs in the same manner as in *Sporobolomyces*. Single spore cultures were used throughout so there is no question of culture purity.

The ballistospores of *Itersonilia pyriformis* do not exhibit the yeast-like budding nor do they have the pink color of those of *Sporidiobolus Johnsonii*. Also the ballistospores of *I. pyriformis* are larger than those of *S. Johnsonii*. There is considerable difference in the appearance of the colonies of these two fungi when grown on the same medium. *S. Johnsonii* produces an appressed growth with very few aerial hyphae. Also, the colony has a brownish cast due to the masses of brown-walled chlamydospores formed. *Itersonilia pyriformis,* in contrast, produces a luxuriant aerial growth, very white and cottony, at least in young cultures. The chlamydospores formed are thin-walled and hyaline. No clamp connections have been observed in association with the chlamydospores in this species.

Itersonilia perplexans Derx is reported (3) to produce "inflated bodies" having clamp connections associated with them. No doubt these inflated bodies, which may be terminal or intercalary, are comparable to the chlamydospores of *Sporidiobolus Johnsonii* and *I. pyriformis*. In *S. Johnsonii* the chlamydospores are thick-walled and brown, being definitely resting spores, whereas, in *I. pyriformis* and *I. perplexans* Derx, they are thin-walled and hyaline. According to Derx (3), there is always a clamp connection associated with the chlamydospore of *I. perplexans*. This is also true of *S. Johnsonii* but not of *I. pyriformis*.

The writer is in agreement with Derx (3) that Stempell (7) probably had a species of *Itersonilia* in culture rather than *Entyloma calendulae* when he described his "Mycel II" and "Halbmondkonidien."

Derx (3) has amended the description of the family Sporobolomycetaceae to include the genera *Tilletiopsis* and *Itersonilia*. The genus *Sporidiobolus,* however, has characters that exclude it from the Sporobolomycetaceae. The presence of chlamydospores in which two nuclei fuse would appear to constitute a sexual phase on the order of that occurring in the smuts. Martin (5) considers the family Sporobolomycetaceae as being composed of imperfect

fungi and consequently classifies it among the Fungi Imperfecti. *Sporidiobolus* with its apparent sexual stage could not, therefore, be placed in this family.

In the genus description of *Tilletiopsis,* provisionally proposed by Derx (2) in 1930 and accepted by him in 1948 (3), no mention is made of chlamydospores or of a sexual stage. However, the writer has isolated two species of *Tilletiopsis* [8] which produce chlamydospores that are strikingly similar to those found in the genus *Entyloma.* There is a good possibility that, when the chlamydospores of these two species are studied cytologically, a sexual phase similar to that in *Sporidiobolus* will be revealed. Preliminary work to date has suggested that such will be the case. If so, *Tilletiopsis* could not logically be included in the Sporobolomycetaceae.

As indicated above, the chlamydospores of *Itersonilia pyriformis* contain a single nucleus. The hyphal cells of this fungus are typically binucleate. If further cytological study reveals that the young chlamydospores are binucleate, a sexual stage such as occurs in *Sporidiobolus* would be demonstrated. In that case, neither *Tilletiopsis* nor *Itersonilia* could be included in the family Sporobolomycetaceae as it now stands.

For these reasons, it is considered premature to include the genera *Tilletiopsis* and *Itersonilia* in the family Sporobolomycetaceae of the Fungi Imperfecti as Derx (3) has proposed.

SUMMARY

1. *Sporidiobolus Johnsonii,* a new genus and species, is described to accommodate an heterobasidiomycetous fungus isolated from a pustule of *Phragmidium rubi-idaei* (DC.) Karst, in a leaf of *Rubus idaeus* L.

2. A name for a previously undescribed species of *Itersonilia* Derx is proposed for a fungus isolated from a dead leaf of *Acer macrophyllum* Pursh.

3. The life histories, physiology, and nuclear conditions of the above fungi are discussed.

[8] The results of this study will be published in a subsequent paper.

4. The validity of the inclusion of the genera *Tilletiopsis* and
Itersonilia by Derx in the family Sporobolomycetaceae is ques-
tioned.

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NOTES AND BRIEF ARTICLES

Taphrina lata Palm

In the writer's recent monograph (Univ. Kansas Sci. Bull. **33**: 3–167. 1949) it is stated regarding certain specimens (in the Stockholm Museum) collected by Palm at Tungelsta, Sweden: "It seems highly probable that this is the material Palm used in describing *Taphrina lata*." This fungus is then reduced to synonymy as *Taphrina carnea* Johanson.

Dr. J. A. Nannfeldt has kindly pointed out the probability of error in this treatment of *T. lata*. A reexamination of the material and of the writer's notes makes it clear that the specimens in question must have been those collected by Palm at Tungelsta and called by him (Arkiv. f. Bot. **15** (4): 1–4. 1917) *Taphrina janus*. (*T. janus* is, by the writer's treatment, synonymous with *T. carnea*.) It could not by any possibility be the type material of *T. lata*.

A further error is the writer's report (l.c.) that Palm described *T. lata* as ". . . affecting only young seedlings a foot or less tall." No such statement occurs in Palm's paper (l.c.). Palm described *Taphrina lata* as affecting individual shoots of *Betula pubescens* Ehrh., causing lateral enlargement and slight thickening of leaves, and enlargement of twigs. He distinguished it further by its broad asci: *asci* 40–45 μ × 18–22 μ; *stalk cells* 16–20 μ × 25–33 μ.

Taphrina lata Palm must be reconstituted as a valid species. Any doubts about it can only be resolved by finding the type material or by collection of material from the type locality.—A. J. Mix, Dept. of Botany, Univ. of Kansas, Lawrence, Kansas.

A Note on Inonotus amplectens Murrill

This very interesting fungus was found and described by William A. Murrill growing on living twigs of *Asimina* in Georgia. On July 28, 1949, Mr. Thomas J. Wesson, Jr., on a field trip found this form growing on the twigs of *Asimina parviflora* (Michx.) Dunal and brought it to the writer who sent it to Dr. J. N. Couch for identification.

On checking the location, this author found this fungus growing on the small papaw along the banks of the Ochlockonee River in Leon county, Florida. The papaws were scattered for 350 feet along the sides of a trail through the dense woods. The fungus occurred on approximately two-thirds of the scattered plants noted. There were 1–4 fruiting bodies per plant.

In a measured space, 15 by 24 feet, 25 knee-high shrubs were counted with an average of 2 thalli per plant. In a second measured area, 15 by 15 feet, 12 waist high papaws were counted with 2–3 fungi per plant.

Inonotus amplectens Murrill usually occurs in the fork of two branches and is attached to both adjacent branches, or to a branch and a leaf petiole. Only occasionally have we found it attached directly to the main stem. Pore surfaces are always oriented so that they project towards the ground. The area of the stem directly under the points of attachment is usually darkened considerably.

It seems to be quite unusual for this fungus to occur in such large quantities. Dr. Herman Kurz who has been collecting in this area for 20 years reports that he has never observed this fungus before this year.—A. W. Ziegler, Dept. of Botany, Florida State University.

MYCOLOGIA

Unexpended reserve, July 1, 1948 .... $ 4,680.69

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The above Mycologia funds are administered by the New York Botanical Garden, and the balances at June 30, 1949, are in agreement with the amounts shown in the financial statements of that organization which have been examined by Price, Waterhouse & Co.

INDEX TO VOLUME XLI *

INDEX TO AUTHORS AND TITLES

* This index prepared by Henry A. Imshaug.

INDEX TO GENERA AND SPECIES

New names, the final members of new combinations, and the page where they appear, are in **bold face type**. Mistakes in the spelling of scientific names encountered in the text are corrected in the index.